PLANT PHYSIOLOGY

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WITH EIGHT PLATES AND SIXTY-SEVEN FIGURES

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TABLE OF CONTENTS

NUMBER 1. JANUARY

		Page
Foreword	The Editors	1
Effects of correlation between vegetative and reproductive functions in the tomato (*Lycopersicon esculentum* Mill.).	Andrew Edward Murneek	3
The extraction of plant tissue fluids and their utility in physiological studies	R. Newton, W. R. Brown, and W. M. Martin	57
Some observations on the growth of yeast	Ellis I. Fulmer	67
Sulphur in rainwater	Scott V. Eaton and J. H. Eaton	77
A plant photometer	Earl S. Johnston	89
Scientific publication	C. A. Shull	90
Notes		93

NUMBER 2. APRIL

Studies on oxygen-supplying power of the soil together with quantitative observations on the oxygen-supplying power requisite for seed germination	Lee M. Hutchins	95
Nutritional studies on *Fusarium lini*	Ernest Shaw Reynolds	151
Adsorption as a means of determining relative hardiness in the apple	Stuart Dunn and A. L. Bakke	165
A study of the clearing of alcoholic plant extracts	W. E. Loomis	179
The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates and potassium in plant solutions	B. E. Gilbert	191
Some further notes on the hydrolysis of starch grains under polarized light	Elizabeth Sidney Semmons	200
A conductivity cell for continuous measurements of respiratory rate.	R. B. Harvey and L. O. Regeimbal	205
Citation of literature	C. A. Shull	206
Notes		209

NUMBER 3. JULY

The mechanism of accumulation of dyes by living cells	G. W. Scarth	215
Evidence on the indispensable nature of zinc and boron for higher green plants	A. L. Sommer and C. B. Lipman	231
Some chemical changes incident to ripening and storage in the Grimes apple	Fisk Gerhardt	257

Science direct

PAGE

Growth studies on fruits.....FELIX G. GUSTAFSON 265
Plant growth-promoting substances, hydrogen ion concentration and the reproduction of *Lemna*.....NORMAN ASHWELL CLARK 273
Differences in resistance to low temperatures shown by clover varieties.....GEORGE STEINBAUER 281
Food storage.....F. M. ANDREWS 287
Notes.....289

NUMBER 4. OCTOBER

Plant physiology at the Ithaca Congress.....FRANK THONE 293
Temperature effects in the metabolism of wheat.....W. E. TOTTINGHAM 307
Sulphur metabolism of yeast.....H. SUGATA AND FRED C. KOCH 337
Studies on the oxidation of certain fatty acids.....J. B. RHINE 349
The distribution of the roots of sugar cane in the soil in the Hawaiian Islands.....H. ATHERTON LEE 363
The digestion of pectin and methylated glucoses by various organisms. HAROLD W. COLES 379
Growth of young wheat plants in auto-irrigated soils, as related to the water-supplying power of the soil and to the adjustment of the autoirrigator. BURTON E. LIVINGSTON, TAKEWO HEMMI AND J. DEAN WILSON 387
The chemical analysis of plant tissues.....C. O. APPLEMAN, W. E. LOOMIS, T. G. PHILLIPS, W. E. TOTTINGHAM AND J. J. WILLAMAN 397
The use of potassium oxalate as a deleading reagent.....W. E. LOOMIS 403
Aqueous extracts of seeds as agents in the preparation of silver sols. E. V. MILLER AND R. P. HIBBARD 409
Climatic effects in the metabolism of maize. W. E. TOTTINGHAM AND H. W. KERR 415
A device for maintaining constant level of culture solutions. F. G. ANDERSON 417
F. G. ANDERSSEN 417

ERRATA, VOLUME I

Page 31, table XI, third column of figures, soluble nitrogen, for 5.14 read 0.514.
Page 34, line 17, for JOHNSON read JOHNSTON.
Page 34, seventh line from bottom, for dut, read due.
Page 53, citation no. 39, for JOHNSON read JOHNSTON.
Page 90, line 21, for portion, read portions.
Page 202, line 13, for huminescence read luminescence.

Vol. I No. 1

PLANT PHYSIOLOGY

JANUARY, 1926

FOREWORD

With this issue, PLANT PHYSIOLOGY takes its place among the American journals published in the interests of botanical science. The editors conceive their task as one of devoted service to the whole field of plant physiology; and it is the hope of those who are guiding the first steps in this service that all plant physiologists may find this journal a useful medium for the publication of important fundamental contributions to our knowledge of plant behavior. We take this occasion to invite constructive criticism and suggestions for the improvement of the journal.

Science is coming to occupy an ever greater and greater place in the affairs of mankind. It exists, indeed, solely for the service of humanity, using the term service in its broadest possible sense; and its chief service is to give a democratic people control over all the forces of nature for the benefit of society as a whole.

Research in plant physiology must proceed in two general directions. It must continue to spread out into the practical fields of human service, such as agriculture, horticulture, agronomy, ecology, pathology, forestry, climatology, etc.; at the same time it must constantly delve more deeply into the problems of developmental metabolism under the leadership of physiologists well trained in the methods of biophysics and biochemistry.

Exploratory research, even if it is more superficial, is of the utmost importance for the practical fields, for it yields us a broader knowledge of the methods of control of plant behavior and plant production. But exploratory research alone, must lead only to empiricism, to rule of thumb methods of practice. Such exploratory research must be followed by an investigation of the fundamental causes of observed behavior. Exploration lays bare the real problems which challenge the highest intellectual endeavors of mankind for their solution. It is evident therefore that these two lines of investigation, practical and fundamental, must always go hand in hand. There can never be a logical separation of these two aspects of our science. Likewise, there can never be a logical separation of the pure

physiologists from the practical physiologists. Our tasks are one, and we must learn to march together in their performance.

This journal, therefore, exists for the general advancement of this great field of investigation, for all of the plant sciences where a physiological attack must be employed in the solution of the problems encountered. It hopes that it may serve as an instrument for the integration and unification of all plant physiologists into a powerful working unit, without interfering with the organized activity of other groups, by providing a central medium through which we can cooperate in the advancement of plant physiology, and of the welfare of plant physiologists, in all fields of endeavor.

To this end it invites the support of plant physiologists of every denomination, "fundamentalists and modernists," pure physiologists and applied physiologists. It has no other purpose, and no other desires than to be of service, and to promote cooperation in the common tasks of advancing plant physiology as a pure and applied botanical science. We extend this invitation with confidence that many hands will lend themselves to lighten the burdens which have been laid upon the editorial committee.—THE EDITORS.

EFFECTS OF CORRELATION BETWEEN VEGETATIVE AND REPRODUCTIVE FUNCTIONS IN THE TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

ANDREW EDWARD MURNEEK*

(WITH ELEVEN FIGURES AND SEVEN PLATES)

Introduction

That a certain antagonism exists between vegetative growth and reproduction seems still to be the rather prevailing opinion among students of plant development. Luxurious vegetation is thought to be detrimental either to the initiation of gametes or to their union, resulting in a conspicuous absence of seeds and fruit, while a limited vegetative growth is considered to be the precursor of marked reproductive activities. In many instances this apparently is true. The facts, however, do not permit one to accept such a view as an axiomatic generalization, for careful analysis of the development of many plants leads to a diametrically opposite conception.

Both empirical and experimental evidence leave one hardly room for doubt that only too frequently a clearly recognizable association does exist between excessive vegetation and lack of fruitfulness on one hand, and diminished growth and abundant seed formation on the other. The question is, however, which in this relationship is cause and which is effect; for our present knowledge of correlation, both quantitative and qualitative, between the various parts or organs of higher plants compels one to accept the existence of a causal relationship.

Data may be marshalled showing the effects of growth on reproduction, but there is also ample proof of exactly the reverse situation. Plants belonging to all three groups—annuals, biennials and perennials—supply a large number of examples where it is clearly evident (1) that vegetative growth is almost quantitatively determined by the developing seeds and fruit; (2) that it often decreases at the exact time and in the exact proportion to the amount of flowers formed and seeds set; and (3) that the rate of vegetative growth is controlled by the developing fruit. These conceptions, though quite contrary to a current point of view, do not appear to be overdrawn. Because of the sequence in time, vegetation usually preceding reproduction, and because of the greater economic importance of seeds and fruit as compared with the strictly vegetative parts of plants, there has been a consistent gathering of proof on the effects of vegetation

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on reproduction, to the seeming neglect of observing and of analyzing the reverse phenomenon, which is apparently just as striking.

The present study was undertaken with the object in mind of determining by means of statistical and chemical analyses the character and extent of correlation existing between vegetative and reproductive activities in the tomato (*Lycopersicon esculentum*). The problem was limited to a single phase, the effect of fruit on vegetative growth.

Review of Literature

A rather careful review of literature at the time of initiation of this investigation (1922) revealed the surprising situation, that, beyond some general considerations (**26**), practically nothing is known of the effects of the developing fruit on vegetative growth of plants. In his unsophisticated enthusiasm the writer thought that he had entered upon an almost virgin and most fertile field of research. The striking results of a preliminary study on correlation between fruit and stem growth in the tomato suggested a renewed scrutiny of all available literature, which resulted in disclosing some valuable information on the subject from rather obscure sources.

As early as 1899 MATTIROLO (**56**)[1] reports in detail a series of most interesting effects of the removal of flowers on growth and development of *Vicia faba*. While normal plants, after maturity of fruit, usually dry up, those from which flowers had been removed daily, showed an excessive vegetative growth of all organs, stems, shoots, leaves, flowers and tubercles (nodules), continuing past the normal season of these plants. This resulted in a total green weight of the plants three times that of the normal ones. Removal of flowers was followed by an abundant branching of the stems, by development of a large number of flowers clear down to the base of the branches, and an increase in amount of tubercles. It is interesting to note that during the period of fruit development the contents of tubercles disappeared, thus establishing a close relation between the two organs. Observation and chemical analysis revealed the fact, that, while in normally growing plants practically all of the tubercles were more or less empty at this time and showed an average nitrogen content of only 4.58 per cent., on deflorated plants they were solid, hard, and held 6.71 per cent. nitrogen. Though no experimental data are given, observation indicated that in leguminous plants there is a periodic emptying of the nitrogenous contents of the tubercles during the time of seed formation. Tubercles having emptied thus, are refilled again after fruiting is over. The following table showing the results with *Vicia faba* may be of interest:

[1] Acknowledgment is due to Dr. J. L. RUSSO of the Department of Romance Languages, University of Wisconsin, for a careful translation of this article.

MATTIROLO—SUMMARY OF RESULTS, *Vicia faba*

	No. of plants	Wt. of shoots	Wt. of roots	Wt. of fruits	Wt. of nodules	Total Wt.	Per cent. N in nodules
		gm.	gm.	gm.	gm.	gm.	
Check	54	3366	917	3408	41.9	6752.9	4.58
Flowers cut	53	9453	2858	7	150.7	12468.7	6.71

MATTIROLO points out the importance of these facts in relation to soil fertility and crop rotation. It is surprising that an investigation and disclosure of such a nature should have passed almost completely unnoticed.

Working with Sea Island cotton MASON (**54, 55**) has recently observed a striking retardation in growth of both the central stem and lateral branches attendant on flower formation and fruit development. The rate of elongation of the central axis decreased at the time of floral development almost in the exact proportion to the amount of flowers formed and fruit set. Coincident with this there was also a marked shedding of the immature flowers, and in older plants even a conspicuous dropping of the young fruits. It is suggested that senescence of the central axis of the cotton plant is associated with a paucity of carbohydrates and probably "other growth promoting substances," as a result of their translocation to the basal fruiting branches. Shedding of flowers and young fruits is attributed to the same cause. Fertilization is assumed to operate in some manner as a causal factor, stimulating the movement of assimilates into the fruit. Very similar results have been obtained with cotton by EWING (**21**). He, too, observed that toward the close of the fruiting season vegetative growth and flowering ceases, all the energy of the plant apparently being utilized in the development of the fruit. But after most of the fruit had attained a mature stage, "second growth" is developed, resulting in new flowers and fruits. In some vigorously growing plants, particularly when the soil is very rich and moist, growth and flowering may be continuous. When flowers were removed at the time of shedding, the plants attained a much larger size and the total number of flowers per plant was nearly doubled.

This information is of assistance in analyzing correctly the extensive statistical records on growth and development of Egyptian cotton made by BALLS and HOLTON (**6, 7, 5**) and of South Sea cotton by HARLAND (**29**). BALLS and HOLTON show that vegetative growth in the cotton plant decreases at the exact time and in inverse proportion to the amount of flowers and fruit present. These results the authors unsuccessfully attempt to explain as being due to environmental factors, particularly a "severe water strain" and the resultant accumulation of "toxins" in the meristematic regions. It is evident that very similar observations may be correctly

interpreted on the basis of correlation, in this case between the developing flowers and fruits and the vegetative organs.

In respect to the successive formation of vegetative parts, buds, flowers, and fruit, the cotton plant and many of the legumes resemble closely the tomato. Hence the above information is particularly significant. In fact, MASON's experimental data and a part of his conclusions, though secured independently and based upon a different plant, are conspicuously similar to those obtained by the writer. This agreement is certainly suggestive of the importance and fundamental nature of correlation in plant growth.

That the depressing effects of fruit on vegetative growth and maturing of floral organs have been recognized in other plants, is indicated by the suggestions of WORK (**78**) that the retardation in maturing of buds in the tomato may be due to the heavy drain on the resources of the plant by the developing fruit. CHANDLER (**11**), too, points out that fruiting has a dwarfing effect on the apple tree, which is shown by a decrease in the diameter of the stem and a marked reduction of the leaf surface of heavily fruiting trees. KENOYER (**41**), has observed a similar reciprocal relation between leaf formation and flowering of many representative species of trees of the Indian monsoon forest.

Materials and Methods

All of the plants used for this investigation were raised from the same lot of seeds of the Bonny Best variety. Having come to the proper stage of development, the seedlings were transplanted from seed beds to three inch pots containing rich soil. Usually a large excess of plants were grown. When they had reached a height of 8–10 inches, at which time the first flower buds were visible, a uniform lot was selected for future use. The plants were lifted from the pots, all soil was carefully removed from the roots by washing in running water and the seedlings at once transplanted to sand cultures. The latter consisted of white quartz sand, free from organic matter, in 10 or 12 inch earthenware pots which were set in granite-ware basins. Two to four plants were grown in each pot. These cultures were kept on low benches in the center of a standard greenhouse.

All transplanting was done as much as possible in the late afternoon or evening, the sand having been previously moistened and the cultures watered once more after setting. If the days immediately following were bright, special protection from the direct rays of the sun was obtained by shading. Watering was adjusted so that the roots received frequent aëration yet the plants were never allowed to suffer from lack of moisture. The temperature of the greenhouse was kept within reasonable limits for tomato culture by means of thermostats and by calsomining of the glass during the summer months.

With these precautions usually very good growth and development was secured. Plants receiving the complete nutrient solution grew almost as well as commercial stock and were identical with such as had been set in pots containing garden soil which was frequently watered with a weak solution of sodium nitrate (see exp. 3). If permitted, some of the plants in sand cultures grew to a height of ten feet and bore a large number of fruits.

The nutrient solutions employed were suggested by Professor E. J. KRAUS. They were made up in the following way:

SOLUTIONS WITH NITROGEN

A	*B*
2 per cent. $MgSO_4$	3 per cent. $CaCl_2$
2 per cent. KH_2PO_4	2 per cent. $CaSO_4$
2 per cent. KNO_3	4 per cent. $Ca(NO_3)_2$

SOLUTIONS WITHOUT NITROGEN

A	*B*
2 per cent. $MgSO_4$	4 per cent. $CaCl_2$
2 per cent. KH_2PO_4	2 per cent. $CaSO_4$
1 per cent. KCl	

In each case the respective stock solutions, A and B, were diluted with six volumes of water before using. For this, as for all other purposes, water from the municipal supply of the city of Madison was employed. It was found to be rather low in nitrogen in any form, but very high in Ca and Mg, particularly in the form of carbonates.

As a rule 500 cc. of the diluted nutrient solution was applied to each pot immediately after transplanting and thence as frequently as it was considered necessary. Usually the solution containing nitrogen was added at five or seven day intervals, that without nitrogen fortnightly or even less frequently. A too copious supply of the solutes was found to result in a high concentration in the lower part of the sand cultures, which would lead to harmful effects on the root system.

In all series of plants two distinct types of growth were obtained. Those receiving nitrogen in the nutrient solution were vegetative and very fruitful. Plants grown without nitrogen were weakly vegetative and able to set only one or two fruits at the most. These two conditions of growth will be referred to hereafter as "nitrogen high" and "nitrogen low" plants. It should be understood, however, that these terms imply only a relative condition in respect to total nitrogen present in the two groups.

A certain standard procedure of treatment of all plants was adopted and rigidly held to throughout this investigation.

1. In practically all instances control or check plants were grown side by side with treated plants in the same pot. This was thought

necessary in order that not only the aerial but also the edaphic environment might be as uniform as possible.

2. Plants in each series were uniformly distributed over the greenhouse benches.
3. By daily removal of axillary growth all plants were trained to a one stem type of growth. This, of course, is easily accomplished with the tomato. The total axillary tissue cut from any plant, attendant on training, in no instance exceeded more than two grams in dry weight, and usually consisted of only a small fraction of this amount.
4. As a rule, all flowers were pollinated daily by transferring the pollen with finger tips to the stigma. Naturally no harm is done in pollinating the same flower more than once. It is thought this procedure facilitated a high degree of fertilization and resulted in the greatest number of fruits that the plant was capable of setting. The importance of this fact will become clear from what follows.

Statistical

Measurements of growth in plants are commonly expressed in terms of dry weight, volume, or height. When the amount or rate of growth is to be ascertained at set intervals of time, manifestly some of the above procedures of measurements are either entirely inapplicable or else very difficult to execute. It is much more simple and convenient to estimate growth by measuring the increase in height. In strictly uniaxial plants or plant organs this method appears to be well within the limits of experimental error, and has been successfully adopted by many investigators (**54, 6, 62, 65, 32**).

For the tomato plants used in the present studies, since they were always trained to a single axis, growth was determined by measuring at weekly intervals the height of the stem from a fixed point at the base to the terminal growing tip. The original records were made to the nearest half centimeter, and the computed averages to the closest millimeter.

In some instances careful data were also secured as to the total fresh and dry weights of stems, leaves, fruits and roots.

Chemical

The material used for chemical analysis consisted of two different types, fresh and preserved tissues. In all cases a representative number of plants were included in each sample.

I. Preserved Material

(a.) *Sampling and preservation.* Sampling was done as much as possible in the early forenoon on a bright day. Plants to be used for analysis

were cut near the ground and fractioned at once into the requisite parts. Where roots were also required, these were carefully lifted from the pots, brushed, washed free of all sand particles under cold running water, and dried between layers of filter paper. The fresh weight having been obtained, the material was cut into small parts, usually fractions of a cubic centimeter, and transferred to a large drying oven with forced ventilation. Temperature of this oven was kept close to 65° C., since LINK and TOTTINGHAM (**49**) have shown that probably the least alterations in the carbohydrate content by enzymatic and respiratory activities take place at this temperature, when forced ventilation is employed. Under these circumstances desiccation was accomplished in 3–7 hours, depending upon the kind of tissues. For still more complete removal of moisture, the material was transferred to a large Freas electric oven at 65° C. for 24–48 hours. The dried material was ground in a drug mill and pulverized in a steel pestle-mill till it passed through a 60-mesh sieve. The powder was preserved for analysis.

(b.) *Ether extraction.* All fruit material was percolated with anhydrous alcohol-free ethyl ether for 18 hours to remove fats, lipoids and other fat-like substances, which might interfere with the extraction and determination of carbohydrates. The ether was removed from the powder by drying for 48 hours at 70° C.

(c.) *Dry weight.* This was obtained by desiccating *in vacuo* to constant weight, 1 gram samples. All subsequent data are expressed on dry weight basis.

A. CARBOHYDRATES

(a.) *Extraction.* Five grams of the powder was extracted with 100 cc. of 95 per cent. alcohol under a reflux condenser for two hours. The temperature of the hot-plate was regulated so as to show but occasional ebullition of the alcohol. While still hot, the extract was separated through a quantitative filter paper into alcohol soluble and insoluble fractions. Both portions were freed from alcohol.

The soluble fraction was cleared with neutral lead acetate, deleaded with a mixture of sodium sulphate and sodium carbonate, neutralized, and brought to a volume of 250 cc.

(b.) *Reducing sugars.* Reducing sugars were determined on 50 cc. portions of the cleared fraction by SHAFFER and HARTMAN'S iodometric method (**71**). The sugar equivalents, in terms of dextrose, were obtained from MUNSON and WALKER'S tables (**79**).

(c.) *Sucrose.* Fifty cc. portions of the above extract were hydrolyzed according to the official hydrochloric acid method in a water bath at 69° C. for exactly 10 minutes, cooled at once in running water, neutralized, and

brought to a volume of 100 cc. The reducing power was determined as in (b).

Repeated attempts were also made to ascertain the possible presence of maltose and other alcohol soluble carbohydrates, that apparently require a more drastic method of hydrolysis. Though several of the methods suggested by DAVIS and DAISH (**16**) and the one used by HARVEY (**31**) for estimation of phloridzin were employed, the results were very inconsistent and hence of little value. Apparently the increased reducing power due to this extra hydrolysis, in this material at least, is often counterbalanced by the destruction of levulose and dextrose.

(d.) *Dextrin and soluble starch.* The alcohol insoluble fraction was extracted at room temperature for one hour with 50 cc. water and frequent stirring. It was then filtered, and the residue washed thoroughly with 90 cc. of water. Ten cc. of hydrochloric acid (sp. gr. 1.125) was then added and the solution hydrolyzed under reflux for 2.5 hours. When cold, it was neutralized, clarified, and brought to a volume of 200 cc. The reducing power was determined on 50 cc. aliquots.

(e.) *Starch.* The residue from the dextrin extraction was washed into a flask and boiled for thirty minutes to insure complete gelatinization of the starch granules. It was then cooled to 38° C., 10 cc. of freshly collected saliva was added, and digested for twenty minutes at this temperature. The mixture was brought once more to boiling, filtered and washed while hot, hydrolyzed, and the amount of dextrose determined as usual.

(f.) *Hemicellulose.* Hemicellulose was analyzed in the residue from starch extraction by washing into flasks with 90 cc. water, adding 10 cc. hydrochloric acid (sp. gr. 1.125), and hydrolyzing under reflux for 2.5 hours. The mixture was cooled, neutralized, clarified, and brought to volume for the usual determination of dextrose.

In every instance analyses were made at least in duplicate and often in triplicate and quadruplicate. All carbohydrates are expressed in terms of dextrose. "Total sugars" represent the sum of reducing sugars and sucrose as determined under the given conditions. "Total soluble carbohydrates" include both the water and alcohol soluble fractions. "Total polysaccharides" are represented by starch and hemicellulose, while "total carbohydrates" is naturally the summation of all determined carbohydrates.

B. NITROGEN

(a.) *Total nitrogen.* Total nitrogen was determined in one gram samples of the air dry powder by the official KJELDAHL-GUNNING method.

(b.) *Nitrate nitrogen.* Three gram samples of the powder were extracted with cold water at room temperature for two hours and filtered through quantitative filter paper. The filtrate was removed to a Kjeldahl

flask, diluted to 250 cc. and nitrate nitrogen estimated by the DEVARDA method as modified by STROWD (**73**). The reliability of this method was tested with pure KNO_3 solution and found to be correct within 0.5 milligram of N.

(c.) *Insoluble nitrogen.* Total insoluble nitrogen was determined by the KJELDHAL method in the residue left after extraction with water, the soluble nitrogen being obtained by difference.

II. FRESH TISSUE ANALYSES

The recent investigations by TOTTINGHAM and his co-workers (**75, 48**) on various methods of preservation and extraction of nitrogenous constituents of plant tissues, point to the fact that practically no method of preservation will leave the soluble nitrogenous fraction of the cells unaltered. Hence in the present study wherever and whenever possible fresh material was employed for analysis of nitrogenous compounds. The method, though very tedious when large numbers of samples are to be used, is very much to be preferred.

(a.) *Extraction.* The fresh material was extracted according to the procedure given by OSBORN (**58, 59**) and CHIBNALL (**12, 13**) and modified in detail by TOTTINGHAM et. al. (**75, 48**). Approximately 100 grams of the fresh material was cut into fine pieces, mixed and used as follows: Fifty grams for extraction and five gram samples for dry weight and total nitrogen determinations (KJELDAHL-GUNNING method).

The 50 gram samples were transferred to large mortars and ground with previously washed quartz sand to a fine pulp. Small amounts of ether were added to facilitate the plasmolysis of cells. The pulp was transferred to lawn cloth on a large funnel and extracted with five or more washings of cold water. The extract was at once filtered through paper pulp in a large Buchner funnel, slight suction being used toward the end, and the filtrate brought to a volume of one liter.

(b.) *Water-soluble nitrogen.* Two hundred cc. aliquots of the filtrate were transferred to Kjeldahl flasks, acidified with sulphuric acid and most of the water driven off. The KJELDAHL-GUNNING method was employed for determining the total nitrogen in the residue, the water insoluble fraction being obtained by difference.

(c.) *Protein nitrogen.* Coagulable protein nitrogen was determined on 200 cc. aliquots of the original filtrate. It was brought to boiling, 2 cc. of 10 percent acetic acid was added and then boiled for three minutes. When slightly cooled, it was filtered and washed through double layers of quantitative filter paper into Kjeldahl flasks. The coagulum was transferred with the filter paper to other Kjeldahl flasks and the total nitrogen determined as usual.

(d.) *Nitrate nitrogen.* In cases where the fresh tissue extracts were analyzed for nitrate nitrogen this was done on the protein free filtrate by the DEVARDA-STROWD method.

(e.) *Amino (aliphatic) nitrogen.* The VAN SLYKE (**34**) method was used for determination of the amino acid content. As the original filtrate was used for this purpose, it undoubtedly represents to some extent other hydrolytic products of proteins besides amino acids, also amide nitrogen and ammonia, if any was present.

(f.) *Hydrogen-ion concentration.* Chopped tissues of representative samples were crushed to fine pulp with previously cleaned quartz sand. The paste was then transferred to clean lawn cloth and the undiluted juice squeezed into clean test tubes. The P_H values were determined electrometrically by means of the type K potentiometer. A small closed Bailey electrode was first used, but this was substituted by the open Hildebrand electrode, which gave just as exact results and was far more convenient. The readings in millivolts were translated to the corresponding P_H values by consulting SCHMIDT and HOAGLAND's tables (**70**), corrections being made for temperature but not for barometric pressure.

(g.) *Microchemical.* In the present study only the following limited number of substances were estimated microchemically: Free-reducing sugars, starch, nitrates, and proteins.

The presence of reducing sugars was determined by the Flückiger's reaction in a heated solution of copper tartrate and strong sodium hydroxide. The presence of starch was tested by the familiar iodine-potassium iodide solution. Diphenylamine, in various concentrations of sulphuric acid, was used for the nitrate reaction, while proteins were estimated by biuret and Millon's tests.

Results

Several series of plants were grown with the primary object of getting acquainted with the response of the tomato to treatment, and of developing methods of procedure. Some of these cultures were conducted in the greenhouses of the Oregon Agricultural Experiment Station, others at the Department of Botany of the University of Wisconsin. Valuable information was thus obtained, which made it possible to secure the maximum response in growth and fruit setting from the rather artificial sand cultures.

The following results have been obtained from experiments conducted with the standardized procedure.

EXPERIMENT 1.—This experiment was undertaken largely for the purpose of careful observation of the effects of fruiting on vegetative growth of both nitrogen high and nitrogen low plants. A detailed description will be given

of their behavior. Some preliminary microchemical analyses were also made.

Seedlings of this series were transferred to sand cultures on Oct. 13, 1922, and 90 plants were grown till Feb. 22, 1923. During this period a total of 17 applications of the full nutrient solution, and in addition four applications of a weak (.72 grams per L.) solution of KNO_3 was given to each pot containing the nitrogen high plants. Those grown without nitrogen received 12 applications of the minus nitrogen solution. All cultures showed comparatively good development.

Nitrogen low plants, of course, exhibited very soon the typical signs of nitrogen starvation, successive yellowing and dying and dropping off of the lower leaves, and a very weak development of the tip of the plant. Such terminal leaves as were still succulent had bluish-green veins and a thin papery texture of the mesophyll tissues. As a rule, these plants were able to set but one fruit. There was no further blossoming, all flower buds yellowing and dropping off very early in their development. (Plate I, A.)

Plants receiving an ample supply of nitrogen developed rapidly, had large clusters of blossoms and produced numerous fruit. All leaves remained green clear to the base. Some time after the setting of fruit on the first two or three clusters, these plants became noticeably weakened in vegetative development. Such flowers as had opened, or were still opening, could not be induced to set fruit. There was a striking yellowing and a rapid increase in number of abortive flower buds. Terminal growth was weak, and further extension very slow. In brief, these plants, too, though continuously supplied with a nutrient solution containing a high percentage of nitrogen, began to exhibit early signs of nitrogen deficiency. It should be pointed out that so far as could be judged the fruit developed normally (plate I, B).

On January 10, when plants of both groups had set the maximum number of fruits, a number of plants were deprived of fruits by cutting away all fruiting clusters close to the stem. Wherever it was possible the vegetatively weaker individual was chosen for defruiting, a control plant being left in the same pot.

The response to this treatment was marked indeed. Ten days later all such nitrogen low plants from which fruit had been removed exhibited already comparatively vigorous vegetative development at the terminal end of the stem. New leaves and flower buds were being formed. The flowers opened normally, were pollinated, and set fruit. At the time of the closing of this experiment, Feb. 22, the treated plants had added 10–30 cm. to their height. The new growth was conspicuously more succulent and often of greater diameter than the old portion of the stem. This new region of the shoot had given rise to a fruit (rarely two), which

was now 2–3 cm. in diameter (plate II). These results are particularly striking in view of the fact that naturally no nitrogen was added to these cultures throughout the experiment. Microchemical tests showed that defruiting of nitrogen low plants resulted probably in a slight increase of reducing sugars in the upper region of the stem and a decrease of starch. No nitrates could be detected anywhere.

Control plants, growing in the same medium and carrying one or two fruits, had made no further vegetative development during this period. The terminal growing point in practically all cases had withered, the fruit becoming "tip" as it were (plate III). It is to be noted that where the fruit had attained full maturity and consequently had been removed, usually no new growth appeared. Evidently a condition of complete or almost complete nitrogen exhaustion prevails in these cases incident to growth and development of the fruit.

Defruiting of nitrogen high plants was followed by a really tremendous growth. It appeared as if such plants were starting life all over again. The first sign of the effect, a change to purplish-green color of the youngest leaves and the developing axillary tissues, could be observed as early as three days after the treatment. This was followed by a most rapid development in both terminal and axillary regions. Even an improvement in color and texture of the old leaves had taken place. The top part of the stem exhibited a very vegetative condition, a much greater diameter, the formation of larger and conspicuously greener leaves, and luxurious flower clusters (plate IV, A). Microchemical studies indicated a slight increase in reducing sugars and a rapid increase in nitrates in this region of the stem.

Fertilization of flowers was effected readily on these plants and large clusters of fruit were formed (plate IV, B). As soon as these began to develop, they in turn inhibited vegetative growth of the new region. It is a curious fact that an exceptionally large number of adventitious root primordia were initiated in the basal region of the new growth. Morphologically their inception could be traced back to the pericycle or probably cambium.

Control plants growing side by side in the same pots showed striking retardation in vegetative growth and reproductive functions during the same time. Often they were only about half or less than half the size of the treated plants, and had but few or no flowers. In many cases all flower buds turned yellow and aborted. Terminal growth had ceased (plates IV and V). No reducing sugars nor nitrates could be found in the terminal portion of these stems. Even a cursory examination made it quite clear that in these plants, too, a close correlation existed between the developing fruit and vegetative growth. Moreover, it was clear that the decrease in

terminal development was in inverse proportion to the amount of fruit present at any particular time, and to their proximity to the growing region. When a proportion of the fruits matured and were removed, particularly from the upper region, growth recommenced and proceeded in this case also in inverse proportion to the number of fruits remaining on the plant and their nearness to the tip of the stem.

Since the experiment was conducted during the winter months, the plants were subject to protracted periods of bright and cloudy days. A prolonged exposure to a comparatively low intensity of light resulted in an increased rate of growth of plants in both groups, but particularly those low in nitrogen. Even such individuals as were very low in nitrogen content, and which had therefore ceased to grow, and appeared stunted, began to develop and made considerable progress when cloudy weather set in. It should be remembered that no nitrogen whatever was available to these plants during this period. Any rejuvenescence that was induced by diminished light supply was, however, of very much smaller degree and often insignificant as compared with that resulting from the removal of fruit.

A large number of microchemical determinations showed invariably an increase in nitrates and a decrease in reducing sugars and starch near the growing regions of nitrogen high plants, after protracted periods of cloudy weather, and a reverse situation following a number of bright days. A similar chemical picture was presented also by nitrogen low plants, with the exception that, whether cloudy or bright, no nitrates were present. Apparently, if nitrates were released from other parts of the plant during cloudy weather, which is possible, they were used up as fast as available for the building of new tissues.

EXPERIMENT 2.—In order to obviate any differences in growth and development that may be induced by decreased light, plants in this experiment were grown during the summer months. A selected uniform lot of 120 seedlings was transplanted to the usual sand cultures on March 16, 1923. The weather was very favorable through the season, and particularly uniformly warm and bright during the time of flower formation and fruit development. As a result of the usual supply of the two kinds of nutrient solutions, the lot was soon separated into two, nitrogen high and nitrogen low, groups. Both developed exceptionally well and in all respects were similar to those of the preceding experiment. Therefore a detailed description of their normal behavior appears to be unnecessary.

On May 18, when to all appearance the maximum of fruit setting had been attained, all fruits were removed from one half of the plants in each

group. As the new set of fruits began once more to reduce or inhibit growth, some of the plants were defruited for the second time on July 1. As usual, one or two control plants remained in each pot.

Careful measurements of the linear increase in height of the stem were made at short intervals, beginning with April 20 and ending on July 28, when the experiment was discontinued. Gross weights of the various parts of these specimens were also recorded.

To test the possible effects of cutting of flowers on their subsequent behavior, a number of average plants of both high nitrogen and low nitrogen classes were continuously deflorated beginning with April 20, when the first blossoms had opened. The flowers were cut on the day when they were fully expanded. Subsequently fruit was permitted to develop on some of the nitrogen low plants of this group, which brought about an immediate checking of vegetative growth.

The summarized results of measurements of height of plants of all groups are given in table I. Their graphical representation is found in figs. 1–4. The growth curve for normally fruiting plants of both nitrogen high and nitrogen low groups is very typical. A natural decline in the rate of growth is conspicuous. If the normal growth rate of a tomato plant were to be illustrated by a typical S curve, these graphs would represent the prolonged top end of such a curve, the so-called "autostatic phase." It is interesting to observe that a continuous cutting of flowers made the plants grow at a uniform rate—the curve is almost straight. When such deflorated individuals were permitted to set fruit again their rate of growth declined at once and ran parallel with normal fruiting plants (fig. 3 and plate VI, A).

It is typical that the cutting of fruit was soon followed by a rapid increase in the rate of vegetative growth, which ran exactly parallel with that of deflorated plants. When fruit appeared once more, the rate declined again and now ran parallel with normal fruiting plants. A second cutting of fruit on the same plants was followed again by identical growth response (figs. 1 and 2). The dip in the curves immediately after the dates of defruiting (fig. 2, *a* and *b*) is due to the fact that, for reasons of emphasis, usually the smaller plants were chosen for the treatment.

The characteristic uniformity of the results leaves no doubt of the striking effects of correlation between fruit and vegetative development in the tomato. Moreover, it is difficult to see how the rate of growth may be due to an autocatalytic reaction, such as has been proposed by OSTWALD (**60**), BLACKMAN (**9**), ROBERTSON (**68**) and others (**63, 65**). The typical S curve, so characteristic of the rate of growth of an organism or an organ, may be accounted for on the basis of correlation. In fact, it is possible, as shown in the tomato, to modify it at will. In this respect the criticism by PEARL

TABLE I

EFFECTS OF FRUIT ON GROWTH OF THE TOMATO

SUMMARY OF MEASUREMENTS OF HEIGHT OF PLANTS, IN CENTIMETERS

DATE	APR. 20	25	29	MAY 4	7	12	17	26	JUNE 4	13	22	30	JULY 9	19	28
PLANTS LOW IN NITROGEN															
Normal (check)	30.7	34.0	37.1	40.3	41.4	44.3	45.9	48.7	52.2	54.7	56.1	57.0	57.6	58.1	58.4
Fruit cut once, Normal	31.2	35.3	39.0	41.9	42.8	44.8	47.0	47.2	49.2	50.4	51.2	51.7	51.7	53.3	53.7
5/18 Cut								47.1	56.7	67.1	71.8	72.3	71.7	71.9	72.0
Fruit cut twice, Normal	31.2	35.3	39.0	41.9	42.8	44.8	47.0	47.1	56.7	67.1	71.8	72.3	71.7	71.9	72.0
5/18, 7/1 Cut													73.6	79.0	86.1
Flowers cut	28.9	32.7	36.1	38.6	39.4	42.2	44.2	48.7	57.8	64.2	70.2	74.8	77.7	82.2	86.5
Fruit present											71.2	72.5	75.0	75.5	75.5
PLANTS HIGH IN NITROGEN															
Normal (check)	39.6	49.5	56.5	63.2	64.9	67.3	69.1	71.3	75.5	80.4	85.1	87.9	92.6	98.2	103.9
Fruit cut once, Normal	36.6	46.4	53.0	60.1	62.3	66.3	68.3	76.0	83.0	92.7	99.7	103.0	105.4	109.4	111.5
5/18 Cut								70.3	89.0	113.2	134.8	143.4	155.5	160.3	168.2
Fruit cut twice, Normal	36.6	46.4	53.0	60.1	62.3	66.3	68.3	70.3	89.0	113.2	134.8	143.4	155.5	160.3	168.2
5/18, 7/1 Cut													148.2	173.0	193.4
Flowers cut	35.2	43.5	48.8	55.2	57.7	63.7	69.7	82.6	105.5	127.7	150.1	163.1	181.2	206.5	229.8

(**61**) that two sets of phenomena are not necessarily causally or in any other way fundamentally related merely because they may be described by the same kind of a curve, is entirely valid.

That the normal ripening of fruit is also promptly followed by a distinct development, provided no other fruit is present, is shown in table II and fig. 5. Naturally no such response could be expected from plants very low in nitrogen, if, as a result of the maturing of fruits, a complete nitrogen exhaustion were to take place.

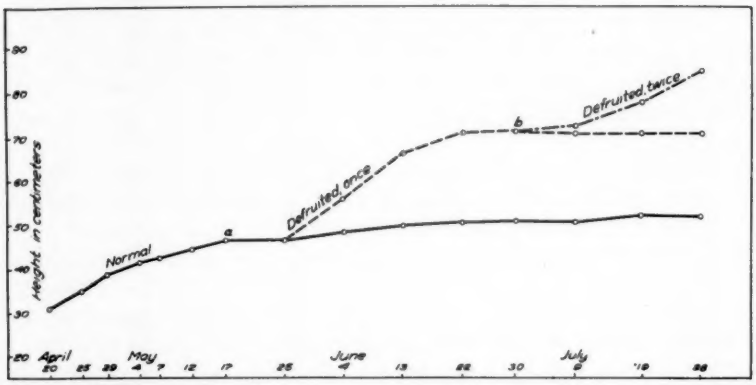

FIG. 1. Differences in growth of normal and defruited nitrogen-low plants. Experiment 2. First defruiting at *a*, second defruiting at *b*.

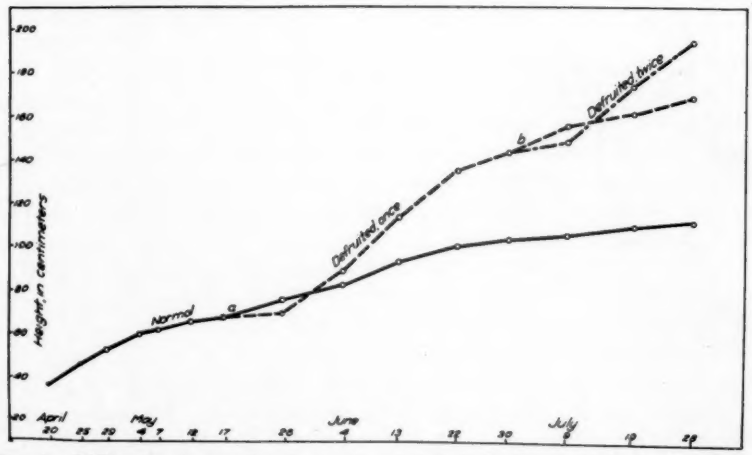

FIG. 2. Differences in growth of normal and defruited nitrogen-high plants. Experiment 2. First defruiting at *a*, second defruiting at *b*.

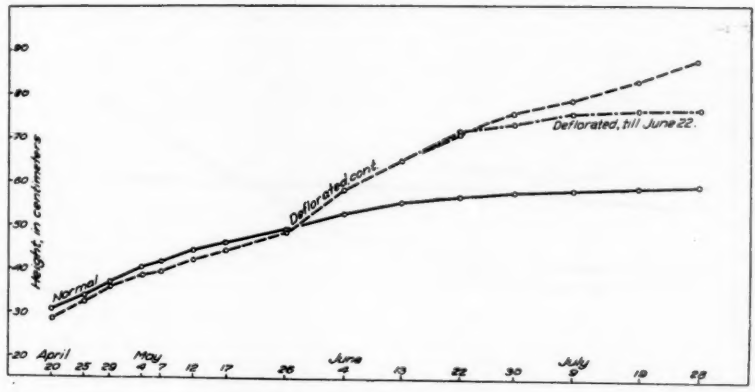

FIG. 3. Differences in growth of normal and deflorated nitrogen-low plants. Experiment 2.

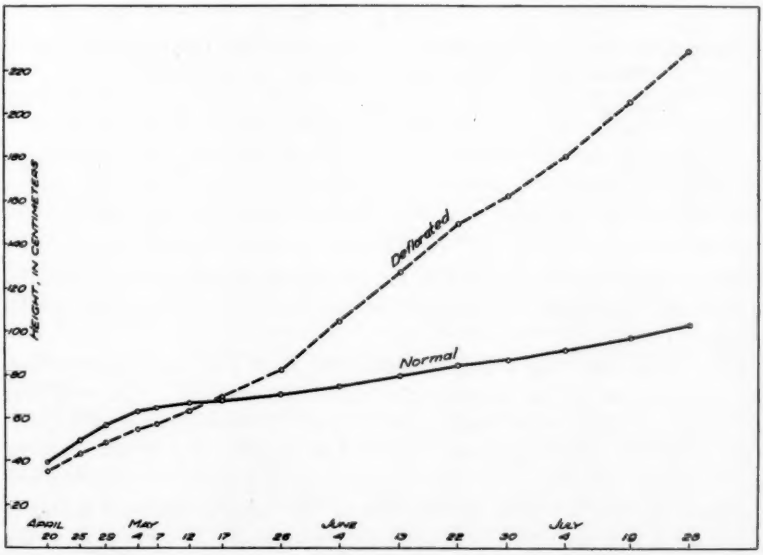

FIG. 4. Differences in growth of normal and deflorated nitrogen-high plants. Experiment 2.

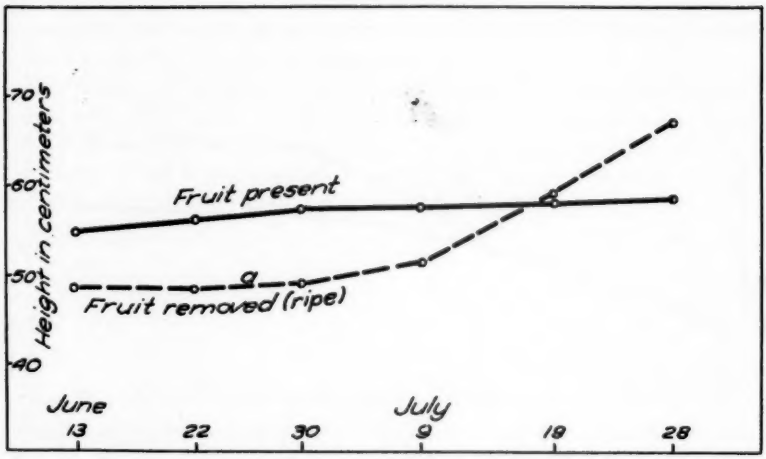

FIG. 5. Differences in growth induced by maturing of fruit, in nitrogen-low plants. Experiment 2. Fruit removed at *a*.

TABLE II

EFFECTS OF REMOVAL OF RIPE FRUIT ON GROWTH OF PLANTS LOW IN NITROGEN. EXPERIMENT 2. MEASUREMENT OF HEIGHT IN CENTIMETERS

DATE	JUNE 13	22	30	JULY 9	19	28
Plants with fruit	54.7	56.1	57.0	57.6	58.1	58.4
Plants with fruit off, ripe, 6/26	48.4	48.5	49.1	51.6	59.1	66.7

The cutting of fruits effected an extraordinary development not only of the stem but practically of all parts of the plant. This is shown clearly in table III.

It is noteworthy that a single removal of fruit prompted the greatest total development of the above-ground parts of the plant. The root system, being insignificant in comparison, is not considered here (see experiments 5 and 6). The total fresh weight of fruits, also, was higher than in normal plants. However, on a physiological or chemical basis these can not be compared with the more mature ones of the check plants. In general, the less fruit was permitted to develop the greater was the growth of stems and leaves. When the nitrogen supply was very limited there was, of course, a loss of a large number of leaves by dropping, which were not included in this record.

TABLE III

TOTAL FRESH WEIGHT OF VARIOUS PARTS OF PLANTS. AVERAGES, IN GRAMS PER PLANT

	No. of fruit per plant	Wt. of fruit	Wt. of stem	Wt. of leaves	Total weight
PLANTS LOW IN NITROGEN					
Normal (check)	1.9	86.0	15.6	6.8	110.3
Fruit cut, once	3.8	93.5	22.5	12.1	131.9
Fruit cut, twice	2.5	38.2	23.5	9.3	73.5
Flowers cut			23.5	12.0	35.5
PLANTS HIGH IN NITROGEN					
Normal (check)	9.5	841.4	52.9	83.6	987.4
Fruit cut, once	20.3	1202.3	160.2	251.0	1633.8
Fruit cut, twice	16.5	620.7	205.4	259.0	1101.6
Flowers cut			319.4	360.1	679.5

EXPERIMENT 3.—This constitutes a parallel lot to experiment 2. These plants likewise were grown during the summer, May 16 to July 28, 1923, and involved a total of 150 specimens. Those of the nitrogen high group were raised in ordinary rich potting soil, instead of sand cultures, for the purpose of testing any possible edaphic effects on the behavior of the plants. In addition they received 500 cc. of the nitrogen-containing nutrient solution at intervals of five to ten days. Naturally the nitrogen low plants had to be grown as usual in sand cultures. The seedlings of this lot were somewhat low in vitality at the time of transplanting, and therefore a large number of plants in the minus nitrogen cultures were not able to set even a single fruit. To obviate this difficulty a small amount of the nitrogen-containing nutrient solution had to be added to pots containing these plants, which naturally brought them to fruiting, a prerequisite for these experiments.

In general the growth responses were identical with those of other series, hence a detailed description is unnecessary. The results emphasized the fact that the sand cultures permitted fully as good a growth and reproduction in the tomato as an ordinary soil medium, within the confines of a ten inch pot. Fruits were cut from the requisite number of plants on July 5, and all were removed for preservation and chemical analysis on July 28.

Differences in growth of normal and defruited plants are given in table IV and fig. 6. Individuals much smaller in size, when deprived of all fruit,

soon overtook and grew to a greater height than those left undisturbed in the pots. This, of course, is identical with previous results. An examination of the data and graphs will show that, in the case of experiments 2 and 3, both nitrogen high and nitrogen low groups, which were selected as the smallest in the lot, after defruiting caught up with the check groups in 12 and 8 days respectively. This is an excellent agreement between the two series.

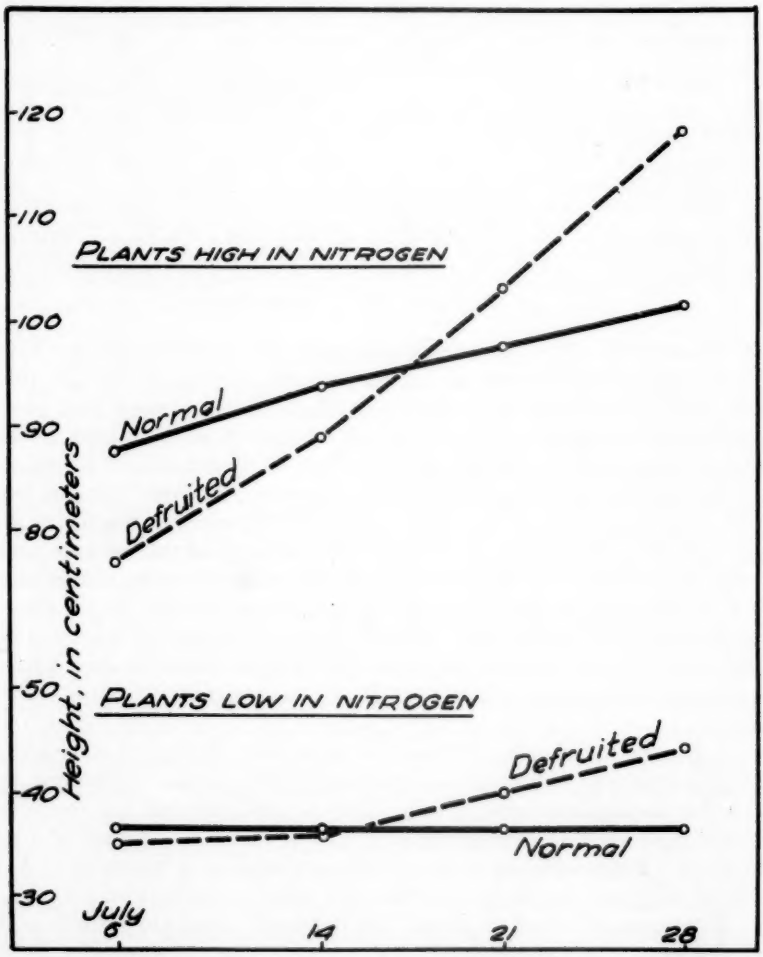

FIG. 6. Differences in growth of normal and defruited plants. Experiment 3.

TABLE IV

EFFECTS OF FRUIT ON GROWTH. MEASUREMENTS OF HEIGHT OF PLANTS, IN CENTIMETERS

DATE	JULY 6	14	21	28
PLANTS LOW IN NITROGEN				
Normal (check)	36.3	36.4	36.4	36.5
Fruit cut, 7/5	35.0	36.2	39.8	44.2
PLANTS HIGH IN NITROGEN				
Normal (check)	87.5	93.7	97.6	101.4
Fruit cut, 7/5	77.1	89.1	102.9	118.2

Chemical analyses of fruit, stems and leaves of the two types of plants were made at the time of defruiting and again at the end of the experiment, July 28. Percentages of the various carbohydrate and nitrogen constituents in terms of dry weight will be found in tables V and VI.

It is noteworthy that defruiting effected a conspicuous increase in practically all of the nitrogenous constituents in the upper part of the stem, but in plants relatively high in nitrogen, in the lower part also. In leaves, however, this increase is quite insignificant. No nitrates to speak of were found in any of the groups. Apparently the supply or the absorption was relatively low, hence nitrates accumulated in neither the stem nor leaves. The proportionally high percentage of nitrogen, particularly of the soluble fraction, is conspicuous in the fruit. But it should be emphasized here that this organ shows also a very high carbohydrate content, largely in the form of reducing sugars and starch.* As one would expect, the removal of fruit resulted in a rapid decrease in almost all forms of carbohydrates, excepting some of the polysaccharides, in the stem of nitrogen low plants. The carbohydrate content of leaves is naturally very variable. Microchemical tests corroborated these results quite clearly, though the amount of reducing sugars appeared to vary within certain limits.

The close agreement between growth response, both vegetative and reproductive, points unmistakably to nitrogen deficiency in the check plants and to a relative abundance of nitrogen in those that were defruited. The external appearance of fruiting plants during the late stages of growth was typically the same as of nitrogen starved plants described and pictured by KRAUS and KRAYBILL (**45**, series O and others) and by WORK (**78**, experiment VI, L), the particular difference being, however, that in the present

* The data on fruit analysis, though based on a mixed material, agree very closely with those of SANDO (**69**).

TABLE V

CHEMICAL COMPOSITION OF NITROGEN LOW PLANTS. DRY WEIGHT BASIS

MATERIAL (Dried at 65° C.)	JULY 4			JULY 28						
				CHECK				FRUIT CUT		
	Fruit	Upper stem	Lower stem	Fruit	Upper stem	Lower stem	Leaves	Upper stem	Lower stem	Leaves
Dry matter	6.55	13.17	16.20	5.60	13.48	15.51	14.72	13.31	17.41	15.24
Total nitrogen	1.445	.495	.44	1.55	.505	.435	1.29	.675	.445	1.42
Insoluble nitrogen	.92	.37	.29	.955	.335	.28	.91	.49	.32	1.05
Soluble nitrogen	.525	.125	.15	.595	.170	.155	.38	.185	.125	.37
Nitrate nitrogen	.00	.00	.00	.00	.00	.00	.00	.00	.00	.02
Total carbohydrates	52.07	33.75	41.43	53.11	—	—	10.79	27.18	39.71	12.78
Total polysaccharides	21.80	17.88	23.66	14.53	—	—	3.00	22.06	27.82	10.65
Starch	14.90	4.55	12.21	7.61	2.30	11.08	.00	5.75	16.04	5.22
Hemicellulose	6.90	13.33	11.45	6.92	—	—	3.00	16.31	11.78	5.43
Total soluble carbohydrates	30.27	15.87	17.77	38.58	7.42	12.89	7.79	5.12	11.89	2.13
Dextrin and soluble starch	5.15	2.52	3.72	1.51	.78	.36	6.79	.91	2.20	1.07
Total sugars	25.12	13.35	14.05	37.07	6.64	12.53	1.00	4.21	9.69	1.06
Reducing sugars	21.56	12.05	8.95	35.75	6.16	6.26	0.16	2.55	5.11	.52
Sucrose	3.56	1.30	5.10	1.32	.48	6.27	.84	1.66	4.58	.54

TABLE VI

CHEMICAL COMPOSITION OF NITROGEN HIGH PLANTS. DRY WEIGHT BASIS

MATERIAL (Dried at 65° C.)	JULY 4			JULY 28						
				CHECK				FRUIT CUT		
	Fruit	Upper stem	Lower stem	Fruit	Upper stem	Lower stem	Leaves	Upper stem	Lower stem	Leaves
Dry matter	5.47	9.97	11.52	5.48	14.13	14.66	13.43	15.25	17.36	13.25
Total nitrogen	2.09	.815	.715	2.09	.605	.60	1.63	1.19	.90	1.98
Insoluble nitrogen	1.17	.54	.48	1.125	.46	.44	1.15	.87	.69	1.45
Soluble nitrogen	.92	.275	.235	.965	.145	.16	.48	.32	.31	.53
Nitrate nitrogen	.00	.00	.00	.01	.00	.00	.01	.00	.00	.01
Total carbohydrates	48.50	24.30	25.90	40.435	22.73	26.84	14.04	14.29	18.39	18.42
Total polysaccharides	19.59	12.69	14.25	12.47	12.30	12.93	9.86	6.92	12.66	14.42
Starch	13.10	1.87	1.89	7.92	2.64	5.10	2.53	1.54	4.34	8.68
Hemicellulose	6.49	10.82	12.36	4.55	9.66	7.83	7.33	5.38	8.32	5.74
Total soluble carbohydrates	28.91	11.61	11.65	27.965	10.43	13.91	4.18	7.37	5.73	4.00
Dextrose and soluble starch	6.43	2.32	2.12	5.00	1.10	2.50	1.88	2.11	1.19	1.22
Total sugars	22.48	9.29	9.53	22.965	9.33	11.41	2.30	5.26	4.54	2.78
Reducing sugars	21.07	4.48	3.93	20.905	4.35	4.32	1.71	2.43	1.08	1.72
Sucrose	1.41	4.81	5.60	2.060	4.98	7.09	.59	2.83	3.46	1.06

experiments such a condition was induced solely by the presence of a relatively large crop of fruits under two widely different planes of nutrient supply. It is evident therefore that *a condition of nitrogen starvation with all its attendant manifestations can be brought about in vegetative parts of the tomato by the correlative effects of the fruit, and quite independently of the external supply of nitrogenous nutrients.* This fact constitutes a very important point in studies of nutrition of higher plants.

EXPERIMENT 4.—This constitutes the largest experiment, involving a total of 480 specimens. The seedlings were transplanted to sand cultures on October 22–23, 1923, and grown till February 4, 1924. Some of the plants were used for experiments as detailed under B to E. All of the groups received the usual treatment, and responded similarly to those of previous cultures.

A. CHEMICAL ANALYSES.

Most of the material in group A was employed for chemical analysis, the latter involving both fresh and preserved tissues. Cutting of fruits was performed on January 8, when retardation in the vegetative development was very distinct. The response was definite and clear cut. Some of the plants were removed for analysis on January 29 and the remainder on February 4. (See table VII and fig. 7.)

TABLE VII

EFFECTS OF FRUIT ON GROWTH. MEASUREMENT OF HEIGHT OF PLANTS, IN CENTIMETERS

DATE	DEC. 28	JAN. 4	11	18	25	FEB. 1
PLANTS LOW IN NITROGEN						
Normal (check)	48.0	50.3	51.7	51.9	.....	52.8
Fruit cut, 1/8	45.4	47.2	48.2	49.0	.....	54.1
PLANTS HIGH IN NITROGEN						
Normal (check)	74.0	75.9	77.1	77.9	79.0	80.4
Fruit cut, 1/8	67.9	68.4	69.6	73.1	81.1	94.2

Data in table VII and their graphical representation in fig. 7 show differences in growth of normal and defruited plants of this group. The results, of course, are identical with those preceding. The forepart of the growth curves of this, as of experiment 3, has been omitted because of its almost absolute similarity to the fully represented curves of experi-

ment 2. While the upward slope of the graphs, showing growth of defruited plants, is typical, it could not be carried forward far enough to make the results more strikingly emphatic, the material having been selected for chemical analysis. A few plants, however, were allowed to develop farther and checked against experiment 2 (tables VIII and IX and fig. 7).

The results of chemical analysis of this series are found in tables VIII and IX. In general, the data agree very well with those of the preceding experiments. Again the removal of fruits caused an increase in the nitrogenous constituents and a decrease in carbohydrates in the terminal half of the stem. The variations in carbohydrates in the nitrogen low plants may be due to the particularly favorable conditions for photosynthesis during the time following defruiting. A conspicuous feature is the comparatively high percentage of nitrates in stems of the nitrogen high plants of both check and defruited groups, thus pointing to an efficient and ample absorption of this constituent from the soil, most likely in excess of the current requirements. That such a situation may exist in many, particularly solanaceous plants, is shown by ANDERSON's (3) work on nitrate reduction.

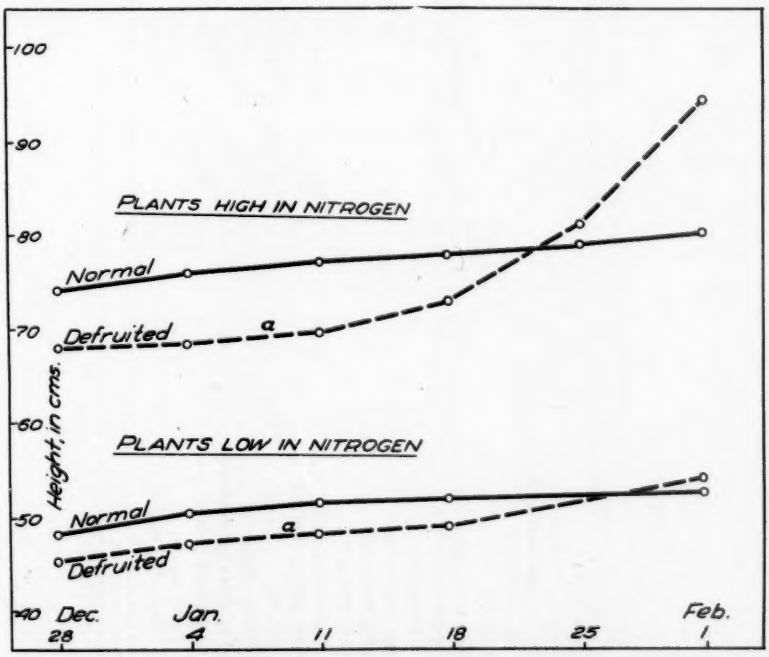

FIG. 7. Differences in growth of normal and defruited plants. Experiment 4–A. Fruit removed at *a*.

TABLE VIII

CHEMICAL COMPOSITION OF NITROGEN LOW PLANTS. DRY WEIGHT BASIS

MATERIAL (Dried at 65° C.)	JAN. 7			FEB. 4					
				CHECK				FRUIT CUT	
	Fruit	Upper stem	Lower stem	Fruit	Upper stem	Lower stem	Roots	Upper stem	Lower stem
Dry matter	6.77	8.69	10.44	7.38	9.95	12.24	12.00	12.19	14.31
Total nitrogen	1.76	.59	.48	1.29	.57	.41	.88	.69	.465
Insoluble nitrogen	1.22	.44	.37	.74	.39	.31	.70	.43	.31
Soluble nitrogen	.54	.15	.11	.55	.18	.10	.18	.26	.155
Nitrate nitrogen	.00	.00	.00	.00	.00	.00	.00	.00	.00
Total carbohydrates	46.60	17.77	.....	45.74	21 87	26.55	30.78	22.52	35.76
Total polysaccharides	22.54	9.13	.....	23.34	14.38	16.87	15.88	14.17	24.15
Starch	15.29	1.89	6.04	15.56	4.49	6.95	6.28	4.45	14.41
Hemicellulose	7.25	7.24	.....	7.78	9.89	9.92	9.60	9.72	9.74
Total soluble carbohydrates	24.06	8.64	11.89	22.40	7.49	9.68	14.90	8.35	11.61
Dextrin and soluble starch	5.29	1.57	4.97	5.35	1.90	2.13	3.44	2.39	3.68
Total sugars	18.77	7.07	6.92	17.05	5.59	7.55	11.46	5.96	7.93
Reducing sugars	18.30	5.50	4.71	17.05	4.60	5.86	5.53	4.08	5.48
Sucrose	.47	1.57	2.21	.00	.99	1.69	5.93	1.88	2.45

TABLE IX

CHEMICAL COMPOSITION OF NITROGEN HIGH PLANTS. DRY WEIGHT BASIS

MATERIAL (Dried at 65° C.)	JAN. 7			FEB. 4				
				CHECK			FRUIT CUT	
	Fruit	Upper stem	Lower stem	Fruit	Upper stem	Lower stem	Upper stem	Lower stem
Dry matter	6.64	7.60	8.95	7.07	8.62	9.51	7.34	11.19
Total nitrogen	2.54	1.84	1.82	2.405	1.615	1.555	1.965	1.64
Insoluble nitrogen	1.28	.76	.73	.93	.61	.60	.88	.66
Soluble nitrogen	1.26	1.08	1.09	1.475	1.005	.955	1.085	.98
Nitrate nitrogen	.00	.89	.86	.00	.85	.85	.76	.71
Total carbohydrates	43.05	18.06	16.72	35.18	18.78	16.62	13.59	20.30
Total polysaccharides	21.16	10.86	10.75	19.87	13.64	11.51	10.90	17.04
Starch	14.53	1.56	1.21	13.90	2.77	1.89	1.38	4.94
Hemicellulose	6.63	9.30	9.54	5.97	10.87	9.62	9.52	12.10
Total soluble carbohydrates	21.89	7.20	5.97	15.31	5.14	5.11	2.69	3.26
Dextrin and soluble starch	6.99	1.78	1.69	6.22	1.17	1.57	1.36	1.76
Total sugars	14.90	5.42	4.28	9.09	3.97	3.54	1.33	1.50
Reducing sugars	13.18	2.22	1.13	8.50	3.38	1.10	.80	.62
Sucrose	1.72	3.20	3.15	.59	.59	2.44	.53	.88

and emphasized by his conclusions, that the presence of nitrates is due largely to differences in rate of assimilation (growth) and absorption from the soil. If the former is slow and the latter rapid, nitrates will accumulate.

It is very interesting that the fruit contained no nitrates. ANDERSON, also, found that while nitrates were present in the stems of many plants (*Physalis Alkekengii, Solanum Dulcamara, S. Lycopersicum,* etc.), no trace of it could be found in the fruit even in early stages of development. Should this be accounted for on the basis of an exceptionally rapid utilization of nitrates for the synthesis of organic nitrogenous compounds in the fruit? In order to throw some further light on this matter, the nitrogenous compounds were fractioned in analyses of fresh material and hydrogen-ion determinations were made (**4**, **27**, **38**). The stems of nitrogen high plants were cut into four parts for analysis in order that a more detailed knowledge of the chemical composition near the terminal end might be secured (tables X and XI).

TABLE X

CHEMICAL COMPOSITION OF NITROGEN LOW PLANTS. DRY WEIGHT BASIS

MATERIAL (Fresh tissue)	JAN. 29–31				
	CHECK			FRUIT CUT	
	Fruit	Upper stem	Lower stem	Upper stem	Lower stem
Dry matter	9.01	11.17	16.07	10.18	14.66
Total nitrogen	1.42	.62	.39	.65	.48
Insoluble N	.92	.33	.235	.375	.30
Soluble N	.50	.29	.155	.275	.18
Protein N	.195	.155	.095	.20	.095
Amino (aliphatic) N	.16	.03	.006	.05	.07

As one would expect, the results show a very high nitrogenous content of the fruit, particularly of the soluble and amino acid fractions, and a greater acidity (P_H). There is a steep gradient of proteins, amino acids, and hydrogen-ion content in the stems. Coincident with the removal of fruit, a marked increase in proteins and amino acids was noted only in the very tip of the stem, while the hydrogen-ion gradient seems to be entirely upset. The data indicate a correlation between the absence of nitrates and a high amino acid and soluble nitrogen content in the fruit. This tends to support the view that probably relatively large amounts of nitrates are rapidly utilized for synthesis in tissues associated with reproduction. Numerous microchemical tests for nitrates and proteins corroborate these results.

TABLE XI

CHEMICAL COMPOSITION OF NITROGEN HIGH PLANTS. DRY WEIGHT BASIS

MATERIAL (Fresh tissue)	JAN. 7					JAN. 29–31								
		STEM					CHECK-STEM				FRUIT CUT-STEM			
	Fruit	Tip	Next to tip	Next to base	Base	Fruit	Tip	Next to tip	Next to base	Base	Tip	Next to tip	Next to base	Base
Dry matter	8.03	7.76	9.45	8.34	10.10	10.38	9.06	9.85	9.95	11.41	6.50	11.66	11.78	13.45
Total nitrogen	.....	2.28	.....	1.72	1.49	1.85	1.47	1.18	1.61	1.65	2.38	1.33	1.52	1.61
Insoluble N	.....	1.648	.....	.980	.544	.67	.735	.815	1.245	1.225	1.18	.86	1.04	.965
Soluble N	1.33	.632	5.14	.740	.946	1.18	.735	.365	.365	.425	1.20	.47	.48	.645
Protein N	.26	.36	.215	.....	.045	.14	.135	.15	.07	.055	.42	.15	.11	.09
Amino (aliphatic) N	.61	.13(↑)	.27	.34	.22	.67	.25	.18	.10	.10	.31	.11	.15	.13
P_H	4.35	5.08	5.22	5.26	5.93	4.20	4.97	5.02	5.05	5.14	5.26	5.33	5.31	5.20

B. Relation to duration of light.

The effects of the relative length of day on growth and reproduction was first clearly demonstrated by Garner and Allard (**23, 24, 25**). Since then, other workers have emphasized its importance (**57, 1, 2**). Hence it was thought of interest to learn to what extent, if any, a drastic reduction of the length of daily exposure to light would influence the effects of correlation between fruit and vegetative growth.

A number of average nitrogen high and nitrogen low plants of experiment 4 were selected for this study. The nitrogen low plants, however, appeared to be too advanced, and therefore had to be discarded. On January 4 the nitrogen high lot was separated into two groups, one being exposed to the normal length of day, the other being kept in a ventilated dark room at approximately constant temperature, excepting that for 2–3 hours (11 to 2 o'clock) each day, they were rolled into the greenhouse on a wheeled table and kept next to the first lot of plants. Immediately preceding the shortening of the day, fruits were removed from one half of the total number of plants in each group. The experiment was continued for 21 days. All of these cultures received the usual applications of nutrient solutions and were watered regularly.

The outcome was rather interesting. Plants of the short day group showed soft succulent tips, yellowish green leaves, smaller flowers, and softer stems. They responded, however, most effectively to defruiting and in a very similar manner to that of the normal long day plants. Checks in both groups behaved as always, fruit reducing growth and inhibiting flowering. That the mechanism of correlation between vegetative and reproductive functions, whatever it may be, was not materially altered by a drastic shortening of the daily exposure to light is clearly evident from table XII and fig. 8, which may possibly indicate its fundamental nature. The records show a prompt response of both short and long day plants to the treatment.

TABLE XII

Effects of short and long days on growth of normal and defruited nitrogen high plants. Measurement of height of plants, in centimeters

Date	Dec. 21	28	Jan. 4	11	25
Long day					
Normal (check)	98.8	105.1	108.4	109.5	111.4
Fruit cut	85.2	87.6	88.6	95.1	112.0
Short day					
Normal (check)	85.4	86.6	90.0	93.0	94.4
Fruit cut	78.5	80.1	83.0	90.6	104.9

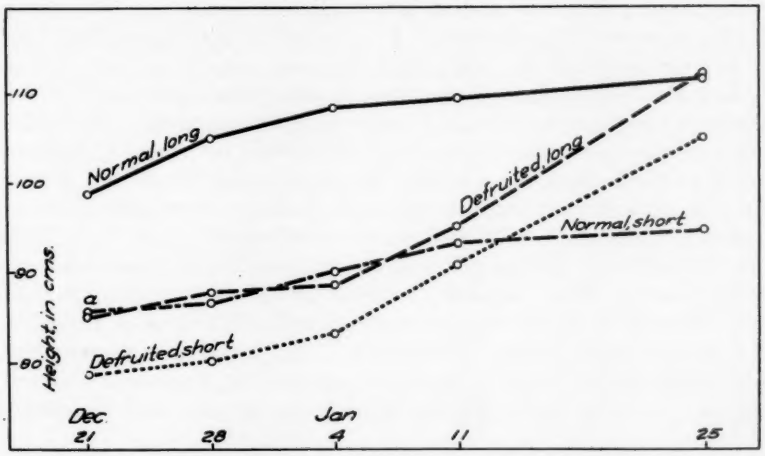

FIG. 8. Differences in growth of normal and defruited long and short day plants. Experiment 4–B. Fruit removed at *a*.

Because of lack of material no quantitative chemical analyses could be made. However, rather careful microchemical tests for reducing sugars, starch, nitrates and proteins, were made of various parts of every type of plant in this experiment.

As a rule, a remarkable stability in approximate amounts of all the substances tested for was exhibited by fruit. This was consistently true of normal plants in both short and long day groups. Such a situation, to be sure, was not presented by the stem. The usual fairly steep descending gradient of carbohydrates, starch, and reducing sugars and the slight ascending gradient of nitrates were almost completely upset ir the stems of short day plants. A distinct reduction in starch and sugars was observed, particularly in the lower half of the stems. This was especially true in specimens that were carrying fruit. At the same time a slight increase in the relative amount of nitrates had taken place. Apparently the developing fruit on the normal, and vegetative growth on defruited short day plants was leading to a rapid exhaustion of the stored carbohydrates, the current synthesis being entirely inadequate. Such an adjustment by the plant could at least be but temporary. This continuing for any length of time would naturally lead either to an absolute or to a relative carbohydrate deficit, which would then operate as an immediate limiting factor in growth.

C. Relation to temperature and intensity of light

In his classical researches on the influence of environmental factors on vegetative and reproductive functions in plants KLEBS (**42**, **43**), among

other things, points to a relation between light intensity and temperature and the character of development. Working with *Simpervivum Funkii* and *S. albidum*, he found that when light is intense temperature may be high without ill effects on flower formation. A diminished light supply with a relatively high temperature will, however, inhibit blossoming. Thus within reasonable limits, not the absolute intensity of light nor degree of temperature is of consequence, but a relation between the two factors. In *Simpervivum* at least, flower formation will not be inhibited when light is reduced even to total darkness, if the temperature remains sufficiently low. Light and temperature, according to KLEBS, are not, however, the immediate cause of such results. These important external factors affect the plant through their influence on the internal carbohydrate complex, leading to alterations of the carbohydrate-nutrient salt relations. This brings us to the more definite carbohydrate-nitrogen relation view, advanced by KRAUS and KRAYBILL (**45, 44**). A discussion of this particular phase, however, will be deferred till later.

More recently JOHNSON (**39**) points to very similar reciprocal relations between light and temperature. Using buckwheat plants as indicators of seasonal variations of climatic conditions in a greenhouse, he found that the retarding effects on growth by radiation may be overcome by increased temperature.

With the object in mind of testing the influence of a low intensity of light and a comparatively high temperature on the development of the tomato, a number of the nitrogen high plants were transferred to a greenhouse where such conditions were obtained. In this particular situation the direct rays of the sun but seldom reached the cultures, and in general the amount of available light was always of low intensity. The temperature was about 25° F. higher than in the greenhouse where a parallel group (A) of experiment 4 was growing. Otherwise the plants were treated identically with those of group A.

The behavior of the two lots (A and C) is compared and expressed in terms of linear growth of the stems in table XIII and fig. 9.

Though the differences in height are very conspicuous, it is doubtful whether a comparison in dry weight or even volume would give the same results. Dut to the very meager light supply and the high temperature, plants in group C exhibited the commonly observed spindling growth, with long internodes and small somewhat yellowish leaves. It is particularly worth noting that most of the flower buds and many flowers, though pollinated daily, aborted on these plants. Consequently there were comparatively few fruits present, a fact which may account, at least in part, for the more rapid elongation of the stems. The abscission of flower buds and

TABLE XIII

COMPARISON OF EFFECTS OF LOW AND HIGH INTENSITIES OF LIGHT AND RELATIVELY HIGH AND LOW TEMPERATURES ON GROWTH OF NITROGEN HIGH PLANTS. MEASUREMENT OF HEIGHT OF PLANTS, IN CENTIMETERS

DATE	Nov. 16	23	30	Dec. 7	14	21	28
Experiment 4—C (Temp.—high. Light—low)...	52.1	67.0	87.9	108.1	119.1	131.9	137.0
Experiment 4—A (Temp.—low. Light—high)...	40.7	47.5	58.2	68.3	73.2	76.1	77.5

apparent sterility must be accounted for on some other basis, for the usual nitrogen high plants, when maturing but a few fruits, behave in just the opposite manner. Most likely the quite prolonged exposure to diminished light resulted in a carbohydrate shortage, the largest part of the available supply being appropriated by the fruits. Under such circumstances naturally the carbohydrates would act as limiting factors in the normal development and functioning of the reproductive organs. A conception of this nature would be more or less in accord not only with the theories of KLEBS but also with the more specific conception of the carbohydrate-nitrogen relations of KRAUS and KRAYBILL (**45**).

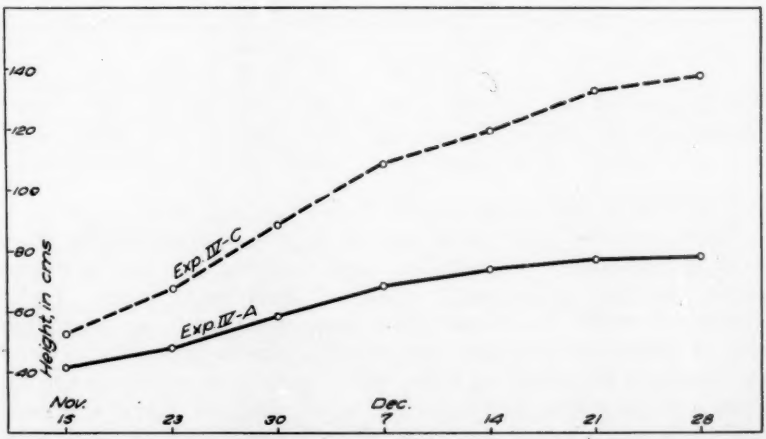

FIG. 9. Differences in growth of nitrogen-high plants induced by relatively low and high temperatures and light intensities. Experiment 4–A, temp. low, light high; experiment 4–C, temp. high, light low.

The evidence from this experiment shows very clearly that in all studies of nutrition, growth, and development, a careful account must be taken of both the qualitative and the quantitative effects of environmental factors on the internal physiological complex of the plant. Though fairly well appreciated, this fact is often lost sight of and appears to require periodic reemphasis.

D. Localization of the effects of correlation

An attempt was made to localize the effects of correlation. A small group of nitrogen high plants were carefully trained to a two-stemmed type of growth. When a large number of fruits had set on both arms, one of them was defruited. The stem from which fruit had been removed began to grow more vigorously and developed new clusters of flowers, which set fruit in due time, while the opposite normal branch of the stem was markedly reduced in vegetation and its buds and flowers dropped off. In this regard the two sides of the plant behaved like individual plants. It should be added that probably more conspicuous differences would have been obtained had the plants not become infected with mosaic toward the end of the experiment (fig. 10, and table XIV).

TABLE XIV

Localized effects of fruit on growth of two-stemmed plants. Measurements of height of each stem, in centimeters

Date	Jan. 15	22	29	Feb. 5	12	19	26
Normal (check) half	47.0	58.0	68.0	76.5	80.5	82.0	84.0
Defruited half	34.5	48.5	61.5	74.0	84.5	87.0	90.0

E. Complete exhaustion of plants by fruit

Plants very low in reserve nitrogen at the time of transplanting, when grown in sand cultures without the supply of nitrogen, frequently become completely exhausted during the process of maturing of fruits. Under extremely favorable conditions, even a single small fruit may accomplish this. A number of such plants were selected from the general lot of nitrogen low plants of experiment 4 for further study. They were grown for a period of ten weeks, during the last six of which no vegetative growth could be observed; instead, a gradual exhaustion of all parts, excepting the fruit, had set in until the plants had lost all their leaves. Finally the stem, too, became exhausted, collapsed, and dried up. Dying of the stem began quite consistently with the part immediately above the ground and pro-

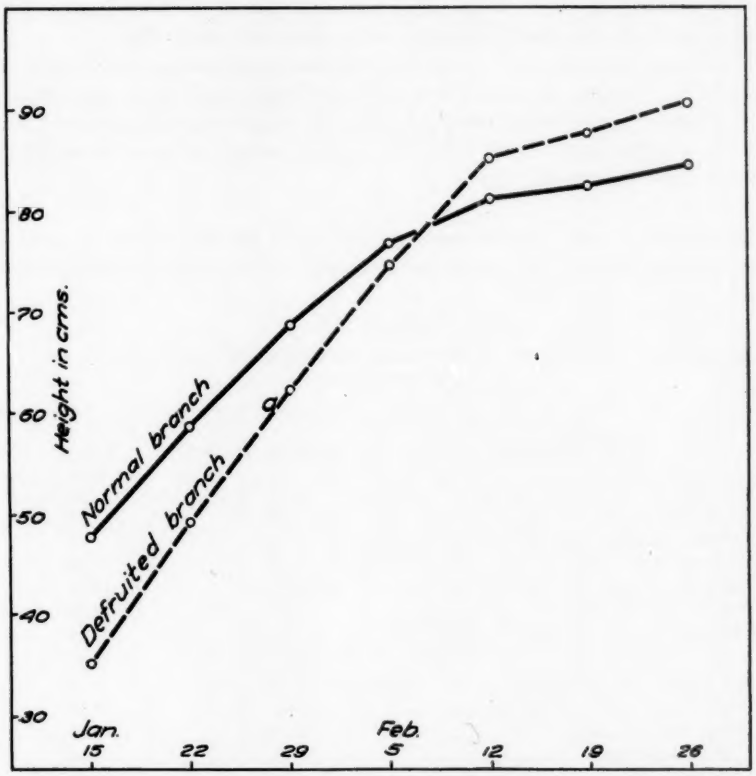

FIG. 10. Differences in growth of the two halves of the same nitrogen-high plant. Experiment 4–D, fruit removed at *a*.

ceeded gradually toward the region of the fruit. Although mature, the latter remained green and succulent till the end, evidently containing practically all the available food supply. A condition of this kind reminds one of a similar exhaustion of many plants of the *Gramineae* and other families during the time of maturing of fruits.

A "post mortem" microchemical analysis showed no nitrates anywhere. There was plenty of starch in the fruit, but none or only occasionally granules in the stems. Reducing sugars were found in abundance in the fruit and to some extent in the stem also. Evidently the latter collapsed before a complete emptying of all reserve carbohydrates had taken place. Much protein was observed in the seeds and in tissues immediately adjoining them.

A number of such completely exhausted plants, without leaves, with dried up stems, but still firm fruits, were analyzed (table XV).

Differences in composition between the fruit and stems are here really remarkable, showing the extent to which the former may drain and exhaust the vegetative parts of the tomato. Thus the soluble fraction especially may be six or seven times as high, and the sugars twenty or more times higher in the fruit than in the stem.

EXPERIMENT 5.—This experiment was conducted for the particular purpose of obtaining material for a chemical analysis of fresh tissues parallel to those

TABLE XV

CHEMICAL COMPOSITION OF COMPLETELY EXHAUSTED NITROGEN LOW PLANTS. DRY WEIGHT BASIS

MATERIAL (Dried at 65° C.)	FRUIT (RIPE)	STEMS
Total nitrogen	2.63	.48
Insoluble nitrogen	1.45	.31
Soluble nitrogen	1.18	.17
Nitrate nitrogen	.000	.000
Total carbohydrate	37.41	17.31
Total polysaccharides	7.66	14.49
Starch	1.08	.66
Hemicellulose	6.58	13.83
Total soluble carbohydrates	29.75	2.82
Dextrin and soluble starch	4.95	1.52
Total sugars	24.80	1.30
Reducing sugars	23.45	1.29
Sucrose	1.35	.01

TABLE XVI

CHEMICAL COMPOSITION OF NITROGEN LOW PLANTS. DRY WEIGHT BASIS

MATERIAL (Fresh tissue)	JULY 14			JULY 29–31				
				CHECK			FRUIT CUT	
	Fruit	Upper stem	Lower stem	Fruit	Upper stem	Lower stem	Upper stem	Lower stem
Dry matter	7.67	17.07	22.05	6.96	16.02	21.12	18.25	21.76
Total nitrogen	1.54	.44	.42	1.31	.42	.40	.55	.44
Insoluble N	.83	.26	.25	.65	.285	.28	.35	.29
Soluble N	.71	.18	.17	.66	.135	.12	.20	.15
Protein N	.22	.11	.09	.29	.07	.05	.09	.05

TABLE XVII

CHEMICAL COMPOSITION OF NITROGEN HIGH PLANTS. DRY WEIGHT BASIS

MATERIAL (Fresh tissue)	JULY 14					JULY 29–31								
						CHECK					FRUIT CUT			
	Fruit	Tip	Next to tip	Next to base	Base	Fruit	Tip	Next to tip	Next to base	Base	Tip	Next to tip	Next to base	Base
Dry matter	8.13	11.45	14.14	15.65	17.99	7.40	14.31	14.82	17.58	18.80	10.41	17.16	18.96	20.50
Total nitrogen	2.37	.95	.72	.86	1.00	2.23	.82	.64	.79	.90	1.72	1.12	1.02	1.20
Insoluble N	.85	.525	.425	.50	.565	.69	.53	.395	.49	.57	1.185	.675	.51	.62
Soluble N	1.52	.425	.295	.36	.435	1.54	.29	.245	.30	.33	.535	.445	.51	.58
Protein N	.49	.235	.18	.16	.18	.54	.185	.18	.19	.16	.20	.20	.135	.11

preceding but based largely on dried material. On June 3–4, 240 seedlings were transferred to sand cultures and as usual grown in two groups, nitrogen high and low plants. Both lots developed satisfactorily. On July 14 and 16 fruits were cut from the required numbers in each group, on which days representative material was also collected for chemical analysis of fresh tissues. The final analysis was made on July 29 and 31 of both treated and normal plants (tables XVI and XVII).

Although this particular experiment was run during midsummer, when the environmental conditions are naturally different than in winter, the chemical data agree remarkably well. Moreover, the percentage composition of all nitrogenous compounds, particularly the soluble ones, is comparatively high in the fruit. But as a result of defruiting a striking increase in the various nitrogenous constituents had taken place in the terminal portion of the stem. These figures agree so closely with those of the preceding experiments, that further discussion is unnecessary.

At this stage of the studies it was thought of interest to determine separately the approximate composition of very young fruits and of seeds and pulp (carpellary wall, fleshy portion of outer integument and placenta) of fully grown ones. Material for this purpose was secured on July 24, when a large number of small fruits, 0.5–2 cm. in diameter, could be gathered. The seed was separated carefully from the pulp of mature fruit, though some of it perforce adhered to the former. Table XVIII contains the data.

TABLE XVIII

CHEMICAL COMPOSITION OF YOUNG FRUITS, AND SEEDS AND FRUIT PULP OF RIPE AND NEARLY RIPE FRUITS. NITROGEN HIGH PLANTS. DRY WEIGHT BASIS

MATERIAL (Fresh tissue)	YOUNG FRUIT	MATURE FRUIT	
		Seeds (Including part of placenta)	Pulp
Dry matter	6.89	8.88	6.50
Total nitrogen	2.19	3.13	1.81
Insoluble N	1.17	1.71	.525
Soluble N	1.02	1.42	1.285
Protein N	.19	.32	.26
Nitrate N	.019	.028	.026

When the chemical composition of young fruits is compared with that of the more mature ones (tables XVI and XVII), it will be noted that the differences are not so conspicuous as one would expect. This may probably

be due to the much higher nitrogenous composition of the pulp in the young specimens. This interpretation is supported by the figures in the two adjoining columns, which show the distribution of the insoluble and soluble nitrogenous fractions in seeds and pulp of more or less fully developed fruit. The seeds seem to contain a much higher percentage of insoluble and total nitrogen.

A table was also prepared to illustrate the approximate calculated distribution of total nitrogen in the nitrogen low plants. The data are self-explanatory (table XIX).

TABLE XIX

APPROXIMATE CALCULATED DISTRIBUTION OF SOME N SUBSTANCES IN NITROGEN LOW PLANTS. JULY 29, 1924. DRY WEIGHT BASIS, PER PLANT

MATERIAL (Fresh tissue)	CHECK				FRUIT CUT		
	Fruit	Leaves	Stem	Roots	Leaves	Stem	Roots
Total weight (fresh), gm.	50.9	12.4	17.4	9.4	16.7	21.2	12.2
Total weight (dry), gm.	3.54	2.29	3.23	1.7	3.34	4.24	1.8
Total nitrogen, gm.	.0465	.0275	.0132	.0070	.0401	.0212	.0090
Percentage distribution of total N	46.60	29.19	14.00	7.21	57.01	30.14	12.85

Careful records on rate of growth and the total yield of various parts of nitrogen low tomato plants were secured, in order that they might be compared with the chemical data. These are summarized in tables XX and XXI and in fig. 11.

TABLE XX

EFFECTS OF FRUIT ON GROWTH. MEASUREMENTS OF HEIGHT OF PLANTS IN CENTIMETERS

DATE	JULY 1	8	14	21	28	31
PLANTS LOW IN NITROGEN						
Normal (check)	52.1	52.5	52.5	52.5	52.5	
Fruit cut, 7/14	53.5	53.9	53.9	54.3	56.2	
PLANTS HIGH IN NITROGEN						
Normal (check)	69.5	75.6	76.8	77.9	79.3	79.8
Fruit cut, 7/16	68.1	73.1	74.6	76.6	84.2	90.1

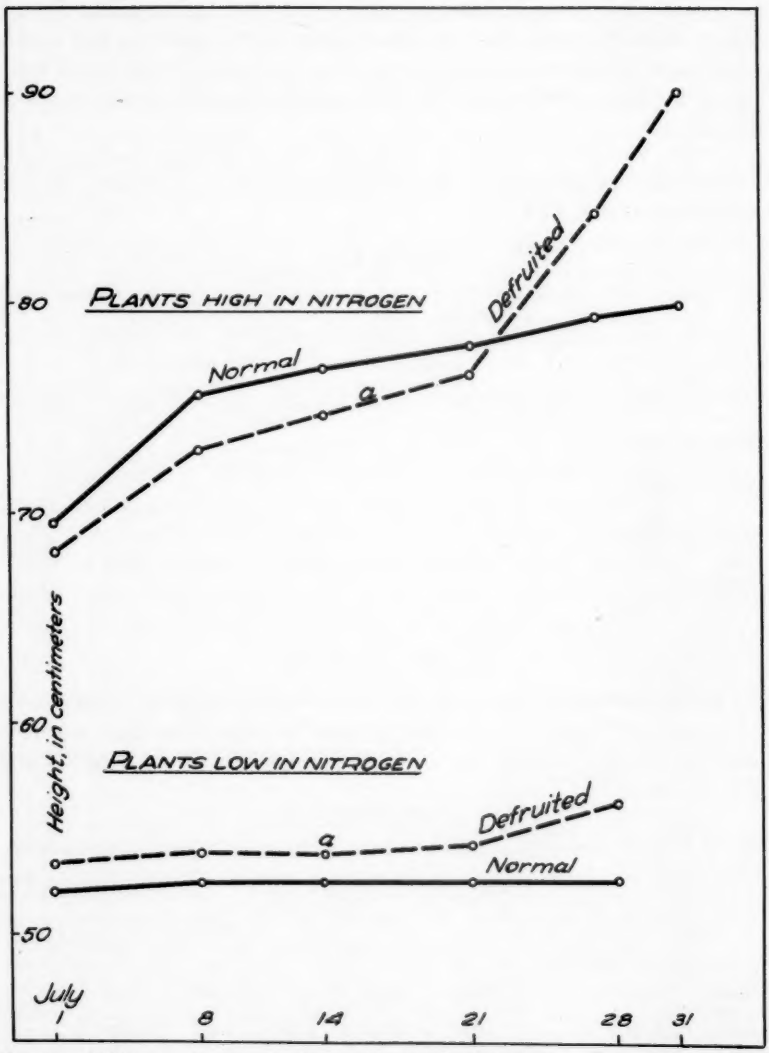

FIG. 11. Differences in growth of normal and defruited plants. Experiment 5. Fruit removed at *a*.

TABLE XXI

TOTAL FRESH WEIGHT OF VARIOUS PARTS OF NITROGEN LOW PLANTS. JULY 29. AVERAGES, IN GRAMS PER PLANT

	Fruit	Stems	Leaves	Roots
Normal (check)	50.9	17.5	12.4	9.4
Fruit cut, 7/14	.....	21.2	16.7	12.2

EXPERIMENT 6.—The object of this experiment was to obtain as vigorous plants as possible, in order to learn whether by this means the effects of correlation could be disarranged or even overcome. Hence at the time when the sand cultures of experiment 5 were started a number of the seedlings were set into a deep greenhouse bed in a very rich garden compost, to which was added from time to time a liberal supply of manure and bone meal.

Growth of these plants was extremely luxurious from the start and remained so for a long time. All of the spurs carried large clusters of blossoms. The usual daily pollination was practiced, resulting in practically every flower developing into a fruit. On July 25, when an average of twenty-five fruits per plant was counted, one half of the group received the usual defruiting. It should be noted that at this time but a slight retardation in vegetative development of the plant could be observed. The supply of all the necessary nutrients appeared to be sufficient for both very vigorous growth and the development of a large crop of fruits. On August 8, twenty-three days later, a different picture presented itself, however. Plants from which fruit had been cut showed an extraordinary terminal and axillary growth. The newly developing stem was several times the diameter of the check plants, the leaves were conspicuously larger and greener, and exceptionally vigorous flower clusters had formed. The development continued in the same direction for another month. On September 10 all check plants, which now carried an average of thirty fruits, exhibited the unmistakable signs of marked retardation in vegetation. The terminal end of the stem became reduced in diameter and carried small rather pale green leaves. The more recent blossoms could not be fertilized, and the comparatively small flower buds turned yellow and abscissed. The striking retarding effects of the fruit were now certain (plate VII).

It is of especial interest that in these particular plants as many as thirty large fruits were required to bring about the same results that were effected by one small fruit on plants very low in nitrogen. Certainly the machinery for absorption of soil nutrients and synthesis of organic substances was ample, and efficient enough to take care of a great number of fruits, but eventually it, too, was overtaxed by the excessive crop. This is particularly

interesting in view of the fact that during this period the plants received several large applications of fertilizers.

That inhibition in vegetative growth could be localized in these highly vegetative plants also, was demonstrated by the behavior of the terminal growth of spurs. On many of the specimens the terminal end of the peduncle of practically all flower clusters extended into a vegetative shoot, which of course developed like any other shoot. But the subtending fruits inhibited its normal development in the usual way (plate VI, B). The interesting feature was, however, that this happened comparatively early in the growth of the plant, at the time when the main stem did not as yet show the slightest signs of retardation. These cases parallel closely those of experiment 4–D.

To show the general distribution of tissues on normal and defruited plants at the end of the experiment, the following table is appended (table XXII).

TABLE XXII

TOTAL FRESH WEIGHT OF VARIOUS PARTS OF VERY VEGETATIVE PLANTS. SEPT. 10. AVERAGES, IN GRAMS PER PLANT

	Fruit	Stems	Leaves	Roots
Normal (check)	4305.0	279.8	907.0	36.5
Fruit cut	.....	756.5	2386.0	135.2

A few of the plants were run through the usual analysis of fresh tissues. Though the stems were sectioned in four parts for this purpose, only the more representative terminal and middle regions were used (table XXIII).

TABLE XXIII

CHEMICAL COMPOSITION OF VERY VEGETATIVE PLANTS. DRY WEIGHT BASIS. AUGUST 8, 1924

MATERIAL (Fresh tissue)	CHECK			FRUIT CUT	
	Fruit	Stem		Stem	
		Tip	Next to base	Tip	Next to base
Dry matter	6.09	6.18	7.31	6.86	8.75
Total nitrogen	2.84	2.40	2.48	3.52	2.72
Insoluble N	.955	1.995	2.205	1.72	2.215
Soluble N	1.885	.405	.275	1.80	.505
Protein N	.421	.29	.20	.39	.19
Nitrate N	.016	1.32	1.76	.85	1.65

The results show much greater extremes in composition than those obtained from plants in the usual sand cultures. One notes that the per cent. of total nitrogen in the fruit and the tips of the stems was practically the same, while in defruited plants it was even much higher in the tip (3.52 per cent.). In the latter instance practically all forms of nitrogen were at a maximum, excepting nitrates. This may account for the extreme growth following the cutting of fruit. Much more insoluble and nitrate nitrogen, but less soluble and protein nitrogen was found in the stems of defruited plants than in fruits of check plants. Again, the usual steep chemical gradient in the stem is not very evident. Moreover, the nitrate content was exceedingly high in the stems (1.76 per cent.), but practically absent (.016 per cent.) in fruit. Thus in this respect, as in all others, the fruit again exhibited an unusual chemical stability (see also tables XI and XVII).

The approximate total distribution of some of the substances was calculated for all parts of these plants and expressed in grams per plant (table XXIV). The relatively high percentage composition in nitrogen of the fruit even under such extremely vegetative conditions is somewhat surprising. In comparison to this the total amount of nitrogen found in leaves is low and in stems and roots almost insignificant. Concurrently with the setting and maturing of additional fruit there is evident a "seasonal" increase in the total amount of nitrogen in tissues associated with reproduction and a corresponding decrease in the vegetative parts. In this respect all of the remaining organs of the plant apparently were profited by the removal of fruit.

Discussion

In the present investigation a somewhat new departure in physiological studies of nutrition of higher plants is introduced. The importance and the rôle of the fruit in the adjustment of a plant to its environment is emphasized. In fact, the fruit, because of its physiological significance, is made the central feature. Judging from the results that have been secured, this seems to be entirely justified.

A careful scrutiny of the foregoing data will disclose certain pertinent facts. It appears to be quite clear that in the tomato vegetative growth is regulated or controlled by the fruit. Under the conditions of the present experiments, this control seems to be determined by two major factors: (1) the number of fruits present on the plant and their proximity to the growing points, and (2) the relative amount of the available nitrogenous food supply. Under conditions of a short daily exposure to light, or a continuous exposure to light of low intensity together with a relatively high temperature, a shortage in carbohydrates may also operate as the immediate limiting factor. Confronted by such a situation, one is led directly to the

TABLE XXIV

APPROXIMATE CALCULATED DISTRIBUTION OF SOME NITROGENOUS SUBSTANCES IN VERY VEGETATIVE PLANTS. DRY WEIGHT BASIS. GRAMS PER PLANT. AUG. 8 AND SEPT. 10, 1924

MATERIAL (Fresh tissue)	AUG. 8				SEPT. 10						
					CHECK				FRUIT CUT		
	Fruit	Leaves	Stems	Roots	Fruit	Leaves	Stems	Roots	Leaves	Stems	Roots
Total weight (fresh)	2122.5	997.5	318.0	34.7	4305.0	907.0	279.8	36.5	2386.0	756.5	135.2
Total weight (dry)	128.1	59.8	21.4	2.5	262.3	54.5	18.8	2.6	131.3	59.0	11.8
Total nitrogen	3.64	1.42	.52	.06	7.45	1.29	.46	.063	3.11	1.84	.368
Insoluble N	1.22	.475	.45	.052	2.51	.434	.394	.055	1.044	1.161	.232
Soluble N	2.42	.945	.07	.007	4.94	.856	.065	.008	2.066	.679	.136
Percentage distribution of total N	64.54	25.18	9.22	1.06	80.40	13.92	4.95	.73	58.50	34.59	6.91

carbohydrate-nitrogen relation concept as proposed by KRAUS and KRAYBILL, the validity of which is indicated by the work of a large number of investigators, who in one way or another have contributed similar evidence (**22, 8, 53, 17, 46, 66, 77, 35, 33, 36, 32**).

There appears to be little doubt that large quantities of nitrogenous nutrients are absolutely necessary for the development of practically all parts of the tomato. Under the category of vegetative tissues come also all the major structures of the fruit, for in this respect it in no sense differs from other rapidly growing organs. If anything, the fruit requires even much more nitrogen. It is also more than probable that for the initiation of floral primordia a preponderance of carbohydrates is prerequisite. Hence a restriction in either the nitrogen nutrients in the soil environment or the stored nitrogenous constituents within the plant would naturally tend to retard development. And because of the effects of a striking physiological control by the fruit, this retardation will be in direct proportion to the amount of fruit present. In extreme cases this will lead to complete cessation of growth as illustrated by all of the nitrogen low plants. Conversely a similar dearth in carbohydrates would inhibit flower formation. This conception is more or less in accord with the results obtained by other workers (**45, 33, 36**).

Tomato plants comparatively low in nitrogen were promptly inhibited in vegetative development by the presence of even a single small fruit. When this was removed, the plant would grow normally, would flower and set fruit once more. Deflorated plants behaved in a similar manner. Those with an abundant supply of nitrogen exhibited even more striking results. In this case, however, the presence of a much larger number, even as many as thirty fruits, were required. Moreover, it is possible to imagine a condition where the supply of nitrogen is extremely large, and carbohydrate synthesis unchecked, which would call for the development of still larger numbers of fruit before a complete restriction in growth would take place. It is apparent, therefore, that in these experiments the behavior of normal fruiting plants has been tested to the limits of one extreme only, the lowest plane of nutrition. The precise boundary line of the other is yet to be established, though it may have been approached quite closely.

Certain detailed features of the effect of correlation between fruit and development may be of interest. When under the respective conditions of nutrition the maximum number of fruits had set, no additional flowers could be fertilized, though careful daily pollination was practiced. This was commonly followed, in the respective order, by a decrease in size of the flower clusters, the yellowing and abscission of the immature flower

buds, and finally by a complete cessation of further growth and even a complete drying up of the terminal end of the stem. All of these signs are very suggestive of a condition of nitrogen starvation of increasing intensity (**45, 78**). Yet in many of the series under consideration the plants were provided with sufficient and even excessive amounts of soil nutrients, as was testified by the extremely rapid recovery and growth of all defruited plants.

The tomato plant apparently does not store any large quantities of nitrogen. Hence with ample pollination a maximum crop under the particular conditions of nutrition is soon attained. The capacity of the plant for absorption and utilization of the nitrogenous constituents seems then to have been reached. Thus a close and delicate balance between absorption and assimilation is probably established. But here is the most interesting feature. The tomato fruit in some way is able to monopolize practically all of the incoming or elaborated nitrogen, thus causing an evident shortage in the strictly vegetative parts of the plant.

An analysis of the chemical data leads to the same conclusions. Coincident with their development, increasing quantities of nitrogen are absorbed and incorporated into the fruit. This statement is supported by several facts. Either no nitrates, or extremely small quantities, were found in the fruit, while at the same time comparatively large amounts of amino acids, soluble and total nitrogen were present (**19**). The fruit showed a very surprising stability in chemical composition even under extreme conditions of nutrition (**10**). When the intake of nitrates is limited, there is naturally none present anywhere in the plant. But when ample supplies are available, nitrates may be found in abundance in strictly vegetative organs, but not in fruits (**64**). Plants extremely low in nitrogen may become completely exhausted (experiment 4–E) as a result of the developing of one or two fruits, practically all of the food supplies being moved and incorporated into the fruit. It is but natural to suspect that under different environmental conditions carbohydrates may also be monopolized by the fruit and their shortage then operates as a limiting factor, with somewhat similar results on vegetation. This, however, has not been definitely established in the present investigation. In every case the fruit contained a much higher percentage of all forms of stored carbohydrates than the stems and roots, but particularly more reducing sugars and starch.

That a very similar situation is exhibited by maize and many other plants may be gleaned from the results of several workers. By extensive chemical investigations HORNBERGER (**37**) was able to prove that the highest absolute amount of non-protein nitrogen is found in maize at the end of fertilization. From then on the path of this form of nitrogen is to the

seeds, where it is changed into protein. This process may go on even when there is a cessation of intake of nitrogen from the soil, thus leading to a reduction of nitrogen in all parts of the plant and an increase in the seeds (fruit). Other investigators (**76, 40, 18, 47**) have shown that at various stages of development the grain of corn and fruits of other plants may contain 50–70 per cent. of the total available nitrogen. Coincident with this concentration in the fruit a conspicuous decrease was found to have taken place in the vegetative parts of these plants. None of these investigators, however, point to the effects of correlation between the developing fruits and vegetative growth.

The question naturally arises as to the mechanism in operation. Coming as it does much later in the sequence of development of the tomato plant, how is the fruit able to establish and exert its dominating influence? Have we here instances of simple physiological mass relations quite similar to that exhibited by *Bryophyllum* (LOEB **50, 51, 52**)? If so, how is this initiated? It is probable that sexual reproduction or gametic union creates a really extraordinary rejuvenescence in tissues closely allied to the developing embryos, thus leading to the establishment of a metabolic gradient similar to that suggested by CHILD (**14, 15**). Still this does not answer the more fundamental question as to how the fruit is able to monopolize practically all of the incoming or synthesized nitrogenous food supply.

Another possibility suggests itself. It is conceivable that plants may have a controlling glandular organization or a system of secretions similar to that existing in animals (**72, 30, 67**). If such an organization of secretions or hormones were to come into play at certain stages of development of particular organs, one would more or less be able to account for the control by and diversion to the fruit of certain food constituents. That plants may secrete hormones has been suggested by several botanists, particularly ERRERA (**20**) and HABERLANDT (**28**). Evidently the basic part of the problem may be accounted for by two possibilities: (1) Either it is a case of localized nutrition brought about by gametic union and the consequent rejuvenescence and initiation of a metabolic gradient, or (2) it may be that we have to deal here with a hormonic control of the physiological substrate, the indispensable chemical, possibly nitrogenous, constituents of the plant. In both instances we enter upon almost completely unexplored domains of plant physiology.

Summary

1. Tomato plants were grown in sand and soil cultures under several planes of nutrition as regards nitrogen supply. In every case a maximum crop of fruits had a strikingly retarding effect on vegetative growth and development. The presence of flowers probably had no influence on growth.

2. This inhibition proceeded in approximately the following order: (1) Destruction of fecundity of the blossoms, (2) decrease in size of floral clusters, (3) yellowing and abscission of flower buds, (4) reduction and cessation of terminal growth of the stem, and (5) complete exhaustion and eventual death of all parts of the plant, excepting the fruit. These signs are typical of a condition of nitrogen starvation of increasing severity.

3. The rate of growth of the tomato, as measured by increments of height, declines at the exact time and in inverse proportion to the amount of fruit set and developing.

4. Chemical analyses and microchemical tests point to nitrogen as an immediate limiting factor effecting these influences of correlation. The percentage composition of nitrogen in the tomato fruit is always higher than in any other part of the same plant. The fruit diverts and monopolizes in some manner almost all the available nitrogen, accounting in part at least for marked carbohydrate accumulation in vegetative structures.

5. Practically no nitrates were found in the fruit, even in extremely vegetative plants, but comparatively large quantities of insoluble and soluble nitrogen were always present.

6. Under certain conditions of nitrogen nutrition, nitrates were found in abundance in the stem and leaves, but none in the fruit.

7. The fruits exhibited higher percentages in all storage forms of carbohydrates, but particularly in starch. But as these were also found in relatively large amounts in other parts, carbohydrates do not seem to have operated as the limiting factors. Conditions are suggested, however, under which this may happen. The carbohydrate-nitrogen relation concept would then come into play.

8. The tomato fruit is conspicuous by its chemical stability under the tested conditions of environment and planes of nutrition.

9. The effects of correlation between the fruits and the growing points may be localized to one half or part of a tomato plant.

10. The influence of the fruit is not neutralized by a short daily exposure to light.

11. Removal of fruits leads in every instance to a complete recovery of the terminal end of the plant and a marked increase in vegetativeness in all other parts. The rate of elongation of the central axis is then identical to that of non-fruiting deflorated plants.

12. When these specimens set fruit once more, vegetative development is retarded again. The rate of growth now runs parallel with any fruiting plant.

13. Stems of defruited plants are very much higher in practically all forms of nitrogen, but especially in the soluble fraction.

14. Suggestions are made as to the possible detailed mechanism of this striking correlation between reproductive and vegetative functions in the tomato.

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EXPLANATION OF PLATES

PLATE I. Normal fruiting plants. Note the typical retardation in development of nitrogen-low plants due to the presence of fruit (A). The characteristic effects of the subsequent behavior of flowers in nitrogen-high plants is shown in B.

PLATE II. Nitrogen-low plants showing the effects of removal of fruit on vegetative development. Observe extraordinary growth of plant *c* as compared with others in the same group (A). Point where fruit was cut at (*x*). B shows depression in the rate of growth of the terminal due to the presence of a fruit (*b*). Compare growth above line in plants *a* and *b*.

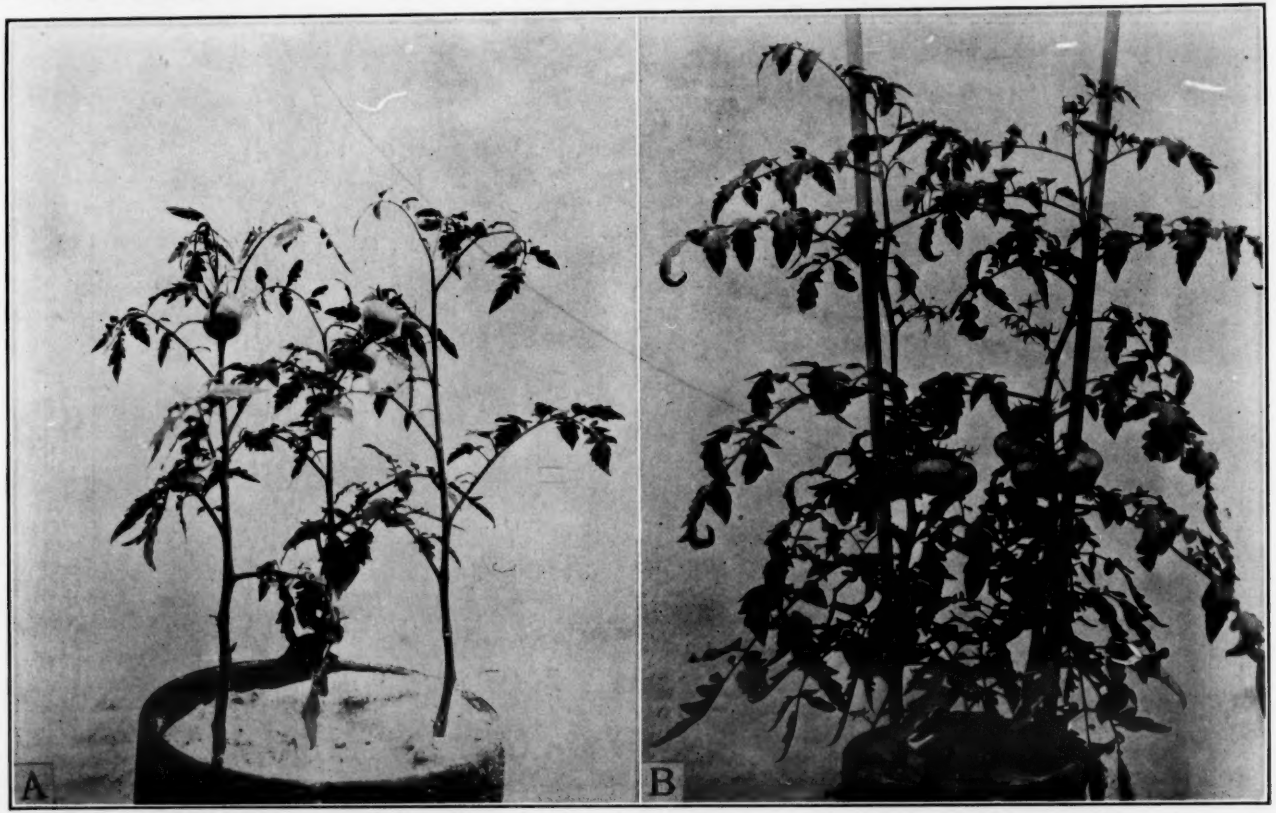
PLATE III. Nitrogen-low plants. A illustrates the effect of cutting the fruit at *x* on behavior of the terminal, plant *a*. B shows how the growing point is often destroyed by the subtending fruit, as at *x*.

PLATE IV. Nitrogen-high plants. The results of removal of two clusters of fruit is illustrated in A. Observe the difference in growth above lines in *a* and *b*. B shows striking inhibition of reproductive functions because of the presence of fruit in plant *a*. Plant *b* (B) was made to set fruit normally by removal of the first two clusters of fruit. Compare development above lines in *a* and *b* (B).

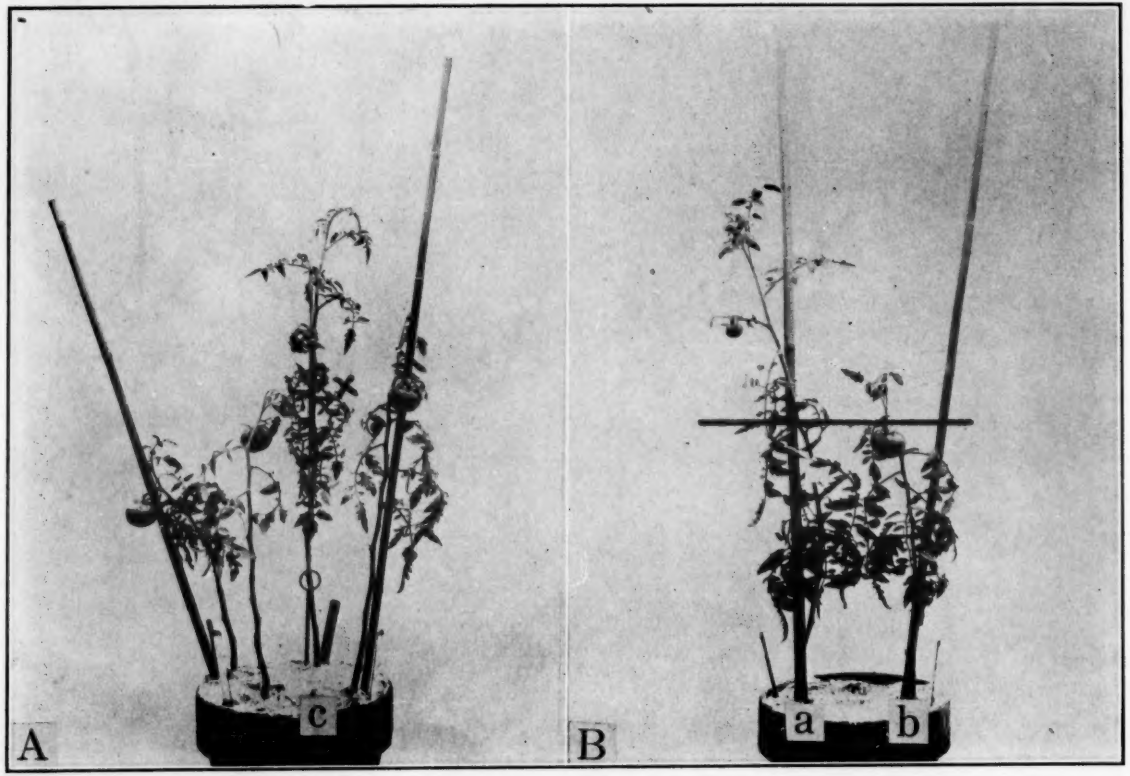
PLATE V. A close view of tips of two plants, showing the effects of correlation on reproductive functions, caused by the absence and presence of fruit on the basal portion. A, fruit cut. B, fruit present. Note setting of fruit in A, *x*, and abscission of flower buds in B, *x*.

PLATE VI. The effects of continuous removal of flowers on vegetative development is shown in plant *c*, A. Compare growth above the lines. B shows that normal development of the vegetative extension of the peduncle is inhibited by the subtending fruit. Observe point of abscission of flower buds at *x*.

PLATE VII. Highly vegetative plants, showing difference in development of defruited (a), and normal plants (b).



MURNEEK—CORRELATION



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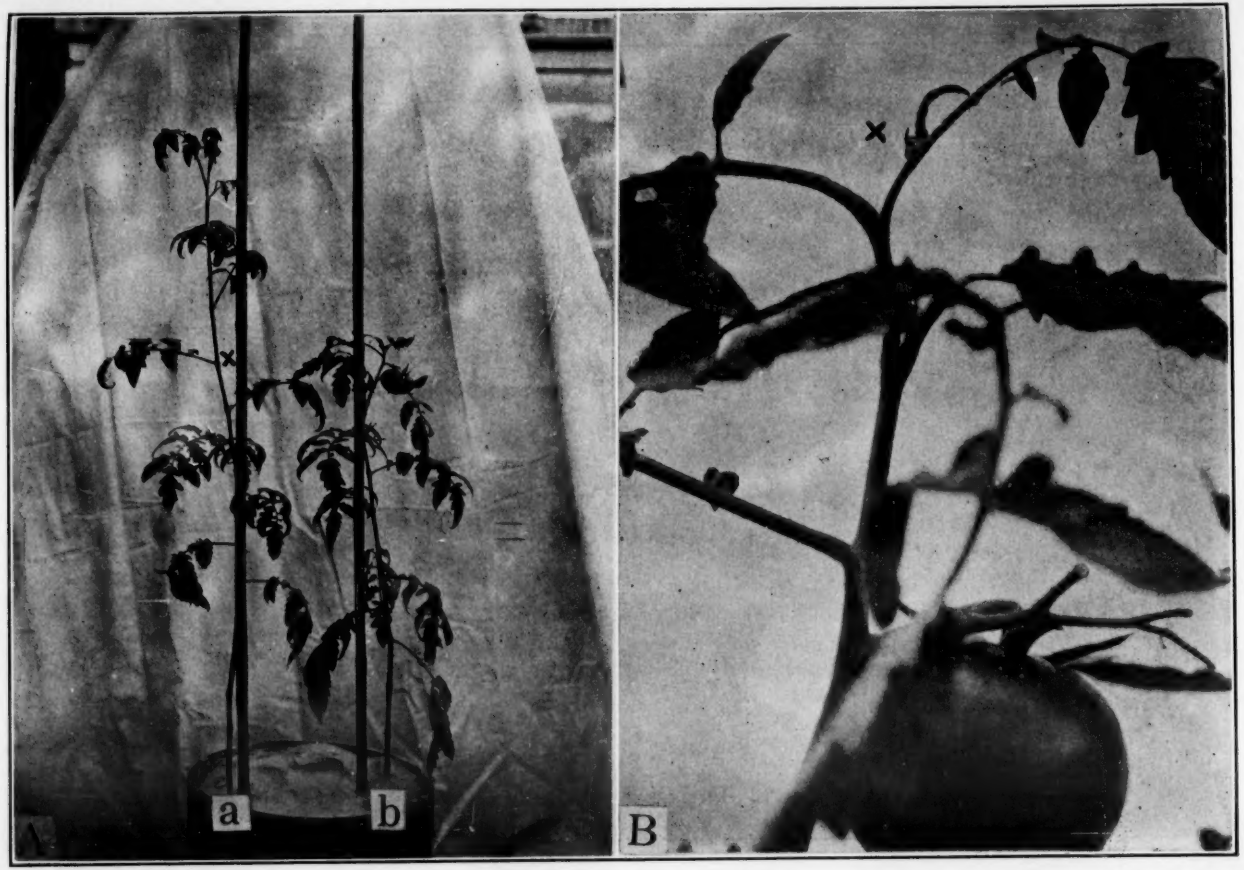
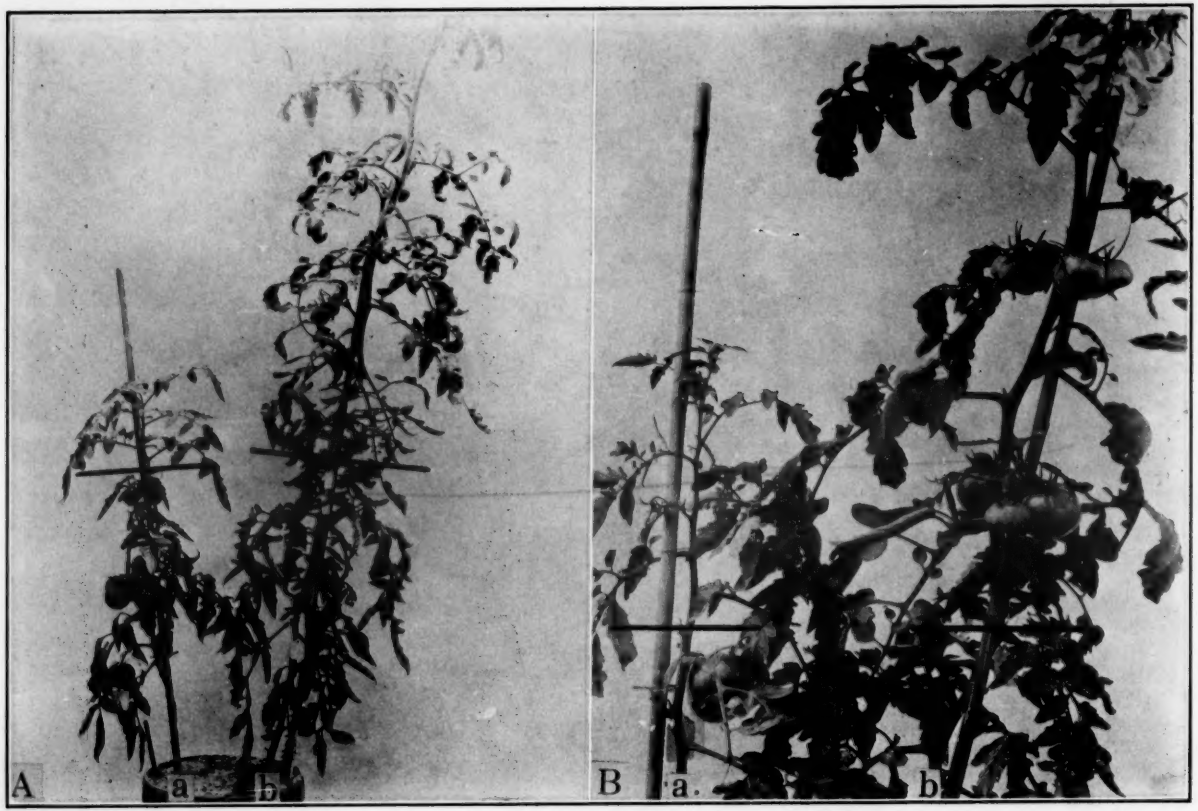
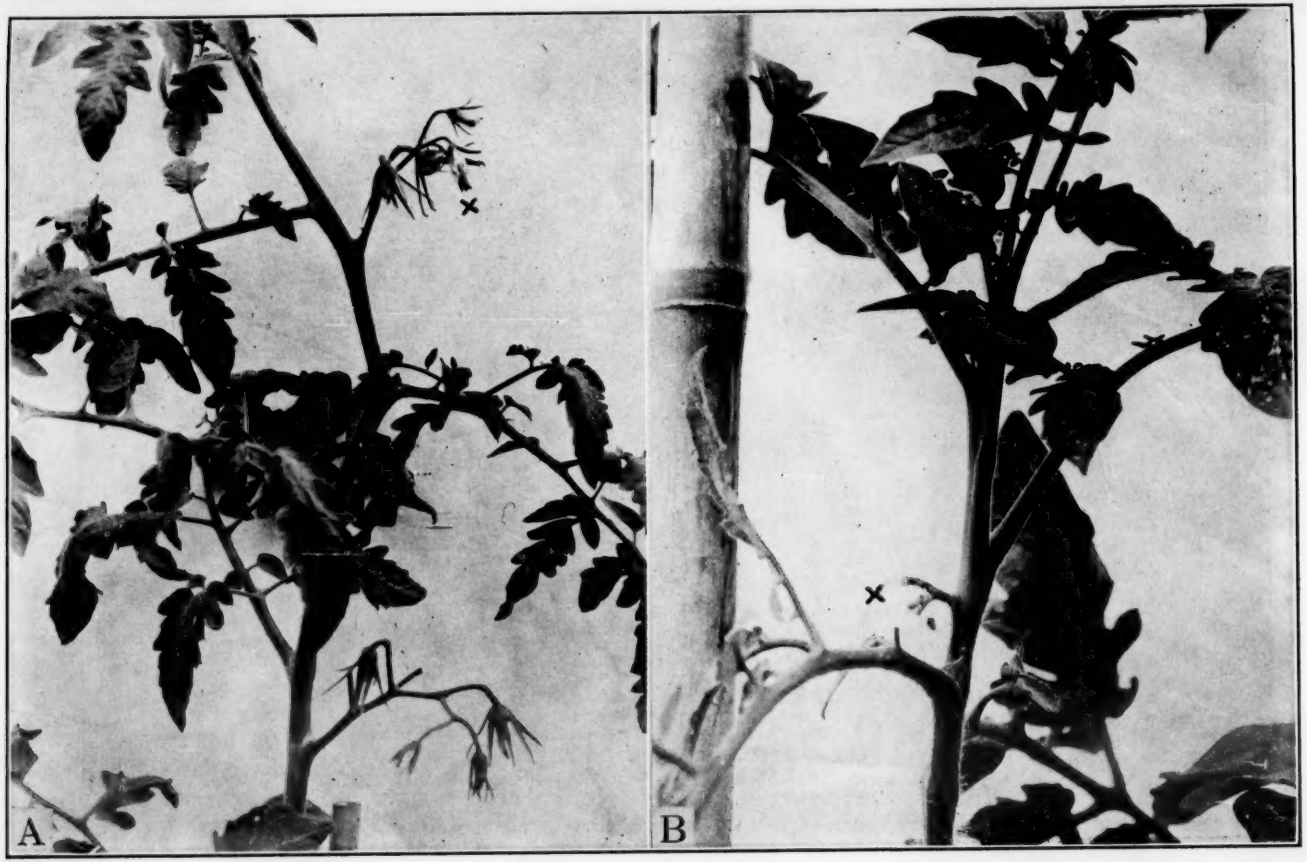


MURNEEK—CORRELATION



MURNEEK—CORRELATION



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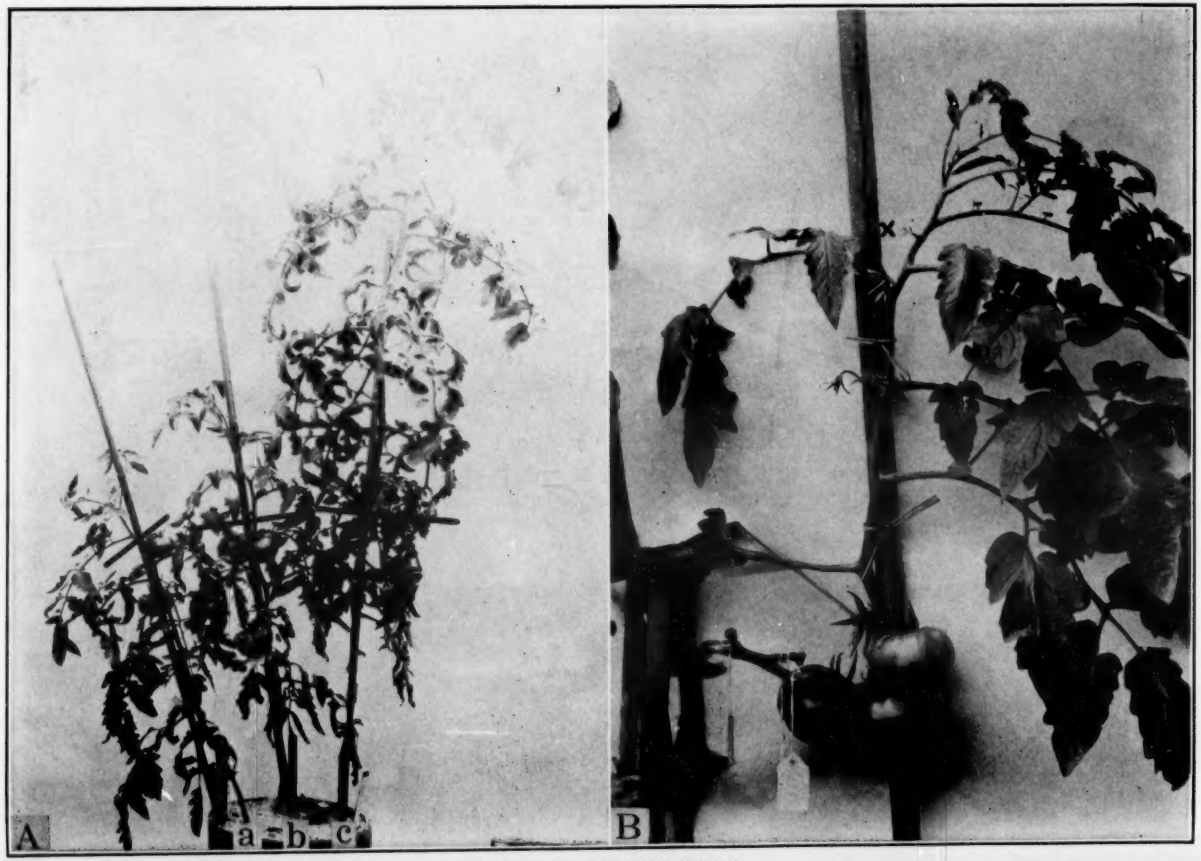

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MURNEEK—CORRELATION

UNIV. OF MICH.

THE EXTRACTION OF PLANT TISSUE FLUIDS AND THEIR UTILITY IN PHYSIOLOGICAL STUDIES[1]

R. NEWTON, W. R. BROWN, AND W. M. MARTIN

(WITH ONE FIGURE)

Introduction

The value of studies of the physico-chemical properties of expressed plant tissue fluids, as indices of physiological and ecological relationships, has long been recognized. Such studies have been confined for the most part to the measurement of osmotic pressure, electrical conductivity, and, more recently, hydrogen-ion concentration. In 1922, NEWTON and GORTNER (**7**) proposed a method for the determination of "bound water," which gives a measure of the relative concentration of hydrophilic colloids. Later, NEWTON (**6**) further extended the series of determinations to include analyses of the press-juice and the parent tissues, from which could be calculated the quantitative distribution of various substances between the tissue fluids and the aplastic or structural portions of the plant.

Both the "bound water" method and the analytical method have been extensively employed in this laboratory during the last few years, in physiological studies of cereals, grasses, and a few other plants. In the course of the work some of the possible errors connected with the extraction of the tissue fluids, and the chances of successfully duplicating results, have been examined. It is believed that the presentation of the results obtained in studying the method of extraction, together with a few considerations as to the utility of the extracted fluids in physiological investigations, will be of interest to a number of physiologists.

Methods

The standard method adopted in the collection of plant material and the preparation of press-juice will first be described. The plant tissue as collected in the field is placed directly in quart Mason jars, packed in crushed ice. When it is difficult to obtain material free from dead leaves, or dead portions of leaves, the jars, after removal to the laboratory, are sorted over one at a time, the plants being removed in small quantities, and the sound portions placed as quickly as possible in another jar, also packed

[1] The work reported herein is part of a project which is receiving the financial support of the Research Council of Canada.

in ice and kept covered except at the moment of transferring material. In the grinding and pressing which follows, all apparatus which comes in contact with the tissues and juice is chilled before use to about 0° C. A portion of 350 to 400 grams of the sound tissue is ground in an ordinary meat-grinder, set to cut as finely as possible. The pulp is wrapped in a single thickness of heavy cotton (previously boiled and rinsed in distilled water), placed in a steel press-bowl 12.5 cm. in diameter, with perforated walls and a gutter base, and hydraulic pressure applied very gradually, just enough to keep the juice flowing slowly but not enough to move the pointer of the pressure gauge away from the zero stop. The pointer actually does not move until a pressure of about half a ton has been reached, equal in terms of unit area of the piston to about 3 or 4 atmospheres. When the pointer does begin to move, no further pressure is applied, nor is any attempt made

TABLE I.

SHOWING AGREEMENT IN SOLID CONTENT AND FREEZING POINT DEPRESSION OF FLUIDS OBTAINED FROM THREE SEPARATE PORTIONS OF ONE COLLECTION OF MINHARDI WHEAT LEAVES

Sample	Solids by refractometer	Freezing-point depression
1	19.1	a 1.379
		b* 1.380
2	19.1	a 1.380
		b* 1.379
3	19.1	a 1.384
		b* 1.380
		c* 1.375

* Replicate determinations with fresh portions of juice.

to secure the fluid remaining in the pulp. From the gutter base of the press-bowl the juice flows through a short length of rubber tubing into a flask packed in ice. The last operation is to whirl the juice 4 minutes in centrifuge tubes jacketed with ice slush, with the rheostat governing the speed of the centrifuge (International No. 2) set at the 8th stop. This removes soil particles and other solids in suspension.

When the conditions under which the fluids are obtained are thus standardized in detail, experimental results may be duplicated without difficulty. An experiment to test this point is reported in table I. Three portions of one collection of winter wheat leaves, collected from a field plot, October 6, 1924, were ground and pressed out separately, and the solid content and freezing-point depression of the juice determined. There was no measurable difference in the three samples.

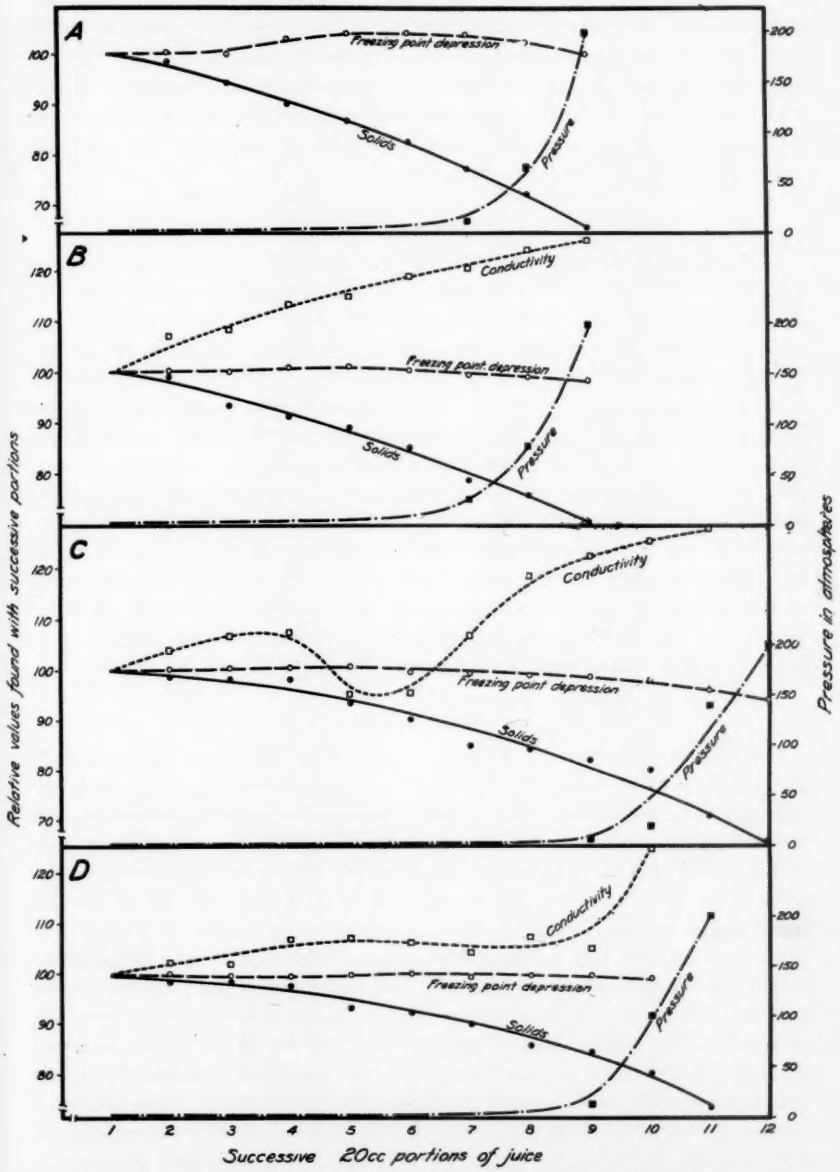

FIGURE 1.—Relative solid content, freezing-point depression, and electrical conductivity of successive 20 cc. portions of juice, collected at gradually increasing pressures. Actual data in corresponding sections A, B, and C–D, table II.

In all the work reported in this paper, the solid content of the juice was determined by the refractometric method proposed by GORTNER and HOFFMAN (3), the freezing-point depression by the Beckmann method, and the electrical conductivity and hydrogen-ion concentration (the latter in one series only) with a highly accurate bridge and potentiometer. The water bath used in the conductivity work maintains its temperature (in these experiments 25° C.) constant within .035° C.

Results

Experimental data on the effect of the pressure used in extracting the fluids are recorded in table II and fig. 1. The standard method was followed in pressing out, except that successive 20 cc. portions of juice were collected separately, and the procedure was extended up to the maximum pressure available. The various constants reported were then determined separately on each 20 cc. portion. In constructing the graph, the pressures are plotted in actual units, but in all other measurements the figures shown in the table for the "1st. 20 cc." have been assigned an arbitrary value of 100, and the values for succeeding portions of juice have been calculated and plotted as percentages of the first figures. The origin of the curve in one case, namely, the freezing-point depression in series C, was fixed by extrapolation, since, as will be observed in the table, the experimental value of the first 20 cc. in this one case was so far out of harmony with all other results that an error seemed probable, and it was considered misleading to base the curve on the uncorrected value.

Discussion

Since three collections of different varieties are involved, the results of the different series, from the point of view of the plant materials used, are not directly comparable. This however makes all the more interesting the considerable resemblance between the curves obtained in all series, as it is evident that their characteristic shapes must be due to the pressures applied. The total solid content decreases from the outset, the rate of decline increasing with the pressure. The freezing-point depression is most nearly constant in value, but nevertheless shows a tendency to rise towards the middle of the extraction process, and fall again when the pressure begins to climb. The conductivity curves, in the three series in which they are available, while they are more irregular than the others, still agree in rising markedly with heavy pressure, and, save for the unaccountable dip in the curve in series C, might almost be said to rise continuously. The tendency for the values of all these properties to change more rapidly after the pressure becomes appreciable, is the justification of our standard practice of

TABLE II

PROPERTIES OF SUCCESSIVE PORTIONS OF JUICE EXPRESSED AT GRADUALLY INCREASING PRESSURES

		1st. 20 cc.	2nd. 20 cc.	3rd. 20 cc.	4th. 20 cc.	5th. 20 cc.	6th. 20 cc.	7th. 20 cc.	8th. 20 cc.	9th. 20 cc.	10th. 20 cc.	11th. 20 cc.	12th. 20 cc.
A. *Einkorn wheat leaves collected June 24, 1924.*													
Pressure in atmospheres		Too small to be recorded on gauge dial						13.	66.	200.	.....	.....	.....
Solids by refractometer		14.6	14.4	13.8	13.2	12.7	12.1	11.3	10.6	9.6	.....	.....	.....
Freezing-point depression		0.993	0.996	0.994	1.028	1.038	1.039	1.036	1.020	0.995	.....	.....	.....
B. *Minhardi wheat leaves collected May 27, 1925.*													
Pressure in atmospheres		Too small to be recorded on gauge dial						27.	80.	200.	.....	.....	.....
Solids by refractometer		22.1	22.0	20.7	20.2	19.7	18.8	17.4	16.7	15.5	.....	.....	.....
Freezing-point depression		1.417	1.420	1.419	1.430	1.435	1.424	1.412	1.405	1.396	.....	.....	.....
Conductivity $\times 10^3$		14.38	15.41	15.60	16.35	16.59	17.17	17.39	17.90	18.13	.....	.....	.....
pH		5.64	5.66	5.51	5.48	5.49	5.50	5.50	5.52	5.52	.....	.....	.....
C–D. *Kharkov wheat leaves collected June 12, 1925.*													
Pressure in atmospheres	C*	Too small to be recorded on gauge dial								7.	20.	140.	200.
	D	Too small to be recorded on gauge dial								13.	100.	200.	.....
Solids by refractometer	C	24.0	23.7	23.6	23.6	22.5	21.7	20.4	20.2	19.7	19.2	17.0	15.8
	D	23.9	23.5	23.5	23.3	22.3	22.1	21.5	20.5	20.2	19.2	17.5	.....
Freezing-point depression	C	1.244	1.287	1.290	1.292	1.295	1.282	1.280	1.275	1.270	1.260	1.232	.....
	D	1.296	1.293	1.290	1.290	1.296	1.300	1.291	1.294	1.292	1.283	.....	.....
Conductivity $\times 10^3$	C	10.11	10.46	10.81	10.89	9.66	9.70	10.84	12.01	12.42	12.67	12.98	.....
	D	10.61	10.86	10.82	11.36	11.40	11.32	11.08	11.43	11.15	13.25	.....	.....

* C – Leaves not frozen before grinding.
D – Leaves frozen with carbon dioxide snow before grinding.

using for analyses only the fluid obtained at low pressure. The use of heavy pressure brings ultrafiltration through the closely packed pulp into play, reducing the colloidal content of the expressed fluid. Possibly some sugar may also be screened out, though our experience with the water-binding capacity of sap colloids suggests that the diminution in the concentration of these substances may be sufficient to account for the observed decline in the freezing-point values. The continued increase in conductivity with pressure shows that the outward passage of electrolyte molecules is not impeded.

Series C and D relate particularly to the influence of pre-freezing of the tissues on the properties of the juice obtained. Different portions of the same collection of wheat leaves were used in the two cases, that used for series C being treated in the usual way, but that for series D before grinding being frozen with a stream of carbon dioxide from a cylinder of the liquified gas until the mass of leaves was filled with carbon dioxide snow. Since the work of DIXON and ATKINS (**2**) this has been a common method of rendering the cell membranes permeable before expressing the fluids. A comparison of the tabular data and curves pertaining to these two series will show that pre-freezing lessened appreciably the deviation of the properties of successive portions of press-juice from those of the first portion pressed out. Nevertheless, the difference in the results of the two series is one of degree rather than of kind. The same characteristic trends are observable in both cases.

Undoubtedly pre-freezing of the tissues is desirable when only the osmotic pressure and conductivity of the fluids are to be determined, though clearly it does not obviate the necessity of standardizing the procedure followed in pressing out. When, however, the object is to obtain the cell contents in a condition as nearly as possible unchanged, pre-freezing is impracticable. Specifically, its precipitating effect on proteins necessitates its omission when the juice is to be used for the study of colloidal properties or protein distribution.

The usefulness of many studies of changes in plant composition, whether seasonal or experimentally induced, has been curtailed by confining the analyses to the entire tissues, whereas it is the tissue fluids which are mainly concerned in physiological activity. When an analysis of the fresh tissue is supplemented by an analysis of the press-juice prepared as described in our standard method, it is possible to calculate the distribution of any desired constituent between the fluid cell contents and the inert supporting framework.

Methods for the complete extraction of leaf cell proteins have been proposed by CHIBNALL (**1**) and by TOTTINGHAM and co-workers (**8**). These,

however, are open to serious objections from our point of view: CHIBNALL's method involves the use of ether, which will bring about changes in the colloids, and both methods require protracted manipulations during which enzyme activity is unchecked. In the method we have adopted, the grinding of the fresh tissues and pressing out of the juice may be completed in a few minutes, and by carrying out these manipulations at a temperature close to 0° C., the conditions are made particularly favorable to the securing of the cell contents in an unchanged condition. It is not attempted to extract all the soluble proteins, but merely to obtain a representative portion of the tissue fluids. Given the composition of the press-juice, including its total solid content, together with the dry matter content of the original tissue, a calculation may readily be made of the total quantity of any fluid constituent in the original tissue.

The assumption that the press-juice has the same composition as the original tissue fluids is of course difficult to prove. An attempt was made to test the point by comparing the freezing-point depression of the leaf tissues, and of the fluid obtained from them. The leaves were wrapped around the bulb of the Beckmann thermometer, and secured in place with rubber bands, or the bulb was inserted in a test-tube containing the ground leaf pulp. The use of a thermocouple, though more convenient in some respects for solids, had been shown in earlier work (**4**) to be less accurate, particularly in ascertaining the degree of undercooling. It was found that determinations with leaves and pulp could not be made with the same precision as with juice, but fortunately the differences proved to be so large as to make probable errors of minor importance. The results are given in table III, the series letters B and C–D identifying the plant materials used with those reported upon in the corresponding sections of table II. The press-juice values in table III are the means of the successive readings in table II up to the point at which pressures begin to be recorded, and beyond which our standard method precludes the further collection of juice.

TABLE III

COMPARISON OF FREEZING-POINT DEPRESSIONS OF FRESH AND PRE-FROZEN LEAVES, GROUND LEAF-PULP, AND PRESS-JUICE

		LEAVES	PULP	PRESS-JUICE
B	Fresh	2.192	1.686	1.424
C–D	Fresh	2.127	.....	1.286
	Pre-frozen	1.805	.....	1.294

The freezing-point depression of the press-juice was in all cases markedly less than that of the leaves, a fact which, considered by itself, would

indicate a lower concentration in the juice than in the original tissue fluids. But it will be noticed that in series B the grinding of the leaves to pulp, and in series C–D the pre-freezing of the leaves, presumably with no change in concentration of the fluids in either case, produced a marked diminution in the freezing-point depression. Evidently the physical organization of the fluids exercises an important influence on the freezing-point. This is not surprising in view of earlier results (**5**, **7**) with plant colloids. Also it is our common experience that determinations of the freezing point of a number of fresh portions of the same lot of juice will check quite closely (*cf.* table I), whereas a second freezing of one portion generally shows a changed value owing to the change in colloidal state produced by the first freezing. When plant tissue is ground and the juice pressed out, the vacuolar sap and protoplasmic colloids, which are separate in the cell, become intimately mixed. Equilibria must be altered, and probably hydrogen-ion concentration changed, with resulting changes in bound water and freezing-point depression. The experimental results given in table III can not therefore be held either to prove or disprove the assumption that the press-juice has the same composition as the original tissue fluids. The point must await further investigation.

Summary

1. By fine grinding of fresh plant tissue and pressing out at low pressure, these operations being carried out close to 0° C., a fluid is obtained which is believed to be of substantially the same composition as the original tissue fluids.

2. The need for careful standardization of the extraction procedure, particularly with regard to the pressure employed, is shown by experimental data.

3. By supplementing ordinary tissue analyses with analyses of the expressed fluids, the distribution of any constituent between the physiologically active and inert portions of the plant may be conveniently determined.

4. The important influence of the physical state of the tissue fluids on their freezing point, and the difficulty of proving the identity of the press-juice with the actual fluids in the plant, are illustrated experimentally.

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SOME OBSERVATIONS ON THE GROWTH OF YEAST

ELLIS I. FULMER

(WITH FOUR FIGURES)

Introduction

In the course of studies by the author and coworkers on the growth of yeast certain general principles of procedure became evident. It is hoped that the formulation of several such observations may be of value in the interpretation of the various reactions of microorganisms to their medial environment. Although the behavior of yeast is used as illustrative material, it may be said that the principles discussed apply to the study of any heterogeneous system whether the dispersed phase be inanimate or animate. It is not claimed that all the rules hereinafter formulated are original with us, but at least it may be said that they have not been so generally recognized as to form a common working basis.

The term "growth" with reference to microorganisms has been used rather loosely and has referred variously to reproduction, increase in weight or volume of organisms in a culture, or to the increase in diameter of a colony, or to the amount of a metabolic product formed. In many instances results obtained by one criterion of growth have been compared to results obtained by other criteria. Obviously it is possible that a culture might grow at a different rate in terms of reproduction than in terms say of gain in weight. In two media there might be the same relative number of cells but their specific gravity might vary so considerably as to give a greater weight in one case than in the other. Similarly there might be a difference in volume of the cells with no accompanying change in number. It would seem then that the increase in weight or volume of cells in a culture or medium may or may not be parallel to the increase in the number of cells. In studies on the growth of microorganisms the criterion of growth should be emphasized, and comparisons based on mixed criteria should be avoided.

Methods

In the material here discussed reproduction is the criterion used. The number of cells is expressed as "count" as determined with the Thoma-Zeiss chamber. The "count" divided by four expresses the concentration of cells in millions per cubic centimeter. In every case the flasks were inoculated with an actively growing culture of *Sacchararomyces cerevisiae* so

that the count was one at the beginning of the experiment. The basal medium termed as medium E (**6**) contains for 30° C. per 100 cubic centimeters, 0.188 gram of ammonium chloride, 0.100 gram of dipotassium phosphate, 0.100 gram of calcium chloride, and 5 grams of cane sugar. It will be shown later that the concentration of ammonium salt for optimum growth must be varied with the temperature used.

Discussion of results

The relative potencies of two materials for the growth of a microorganism cannot be determined by the comparison of the effects of the extracts or solutions of equal weights of dry or basal materials. It will be necessary in each case to determine the optimum concentration in a series before such a comparison may be made.

This point may be illustrated by data previously (**7**) published on the effects of extracts of alfalfa and of wheat embryo upon the growth of yeast. In fig. 1 the yeast crop is plotted as a function of the stimulant added, in terms of per cent. of original material in the form of extract per 100 cubic centimeters of medium. From the diagram it is evident that if investigator A based his results upon the stimulant from extracts represented by 0.06 gram of dry materials per 100 cubic centimeters of medium he would conclude that wheat embryo is more potent in stimulant than the alfalfa; B who used 0.13 per cent. would conclude that the materials are equi-potent; while C basing his results upon 0.2 per cent. would regard the alfalfa more potent than the wheat embryo. As a matter of fact the extract of alfalfa will give greater growth than is possible with any concentration of the wheat embryo extract. The only instance in which two materials could be compared in potency on an equal weight basis would be the very special case in which the effect-concentration curves are parallel at every point. In the instances cited, the curves are not only not parallel but they cross, leading to a reversal of the order of potency. Much work has been done, for instance, on the effect of various nitrogenous compounds on the growth of yeast and comparisons drawn on the basis of the effects of single concentrations. Obviously such results are conclusive only for the particular concentrations tested and no general conclusions are warranted. A great deal of the controversy in the literature regarding the relative advantages of two nutritive materials undoubtedly owes its origin to the neglect of the factors outlined here.

An interesting corollary to the first statement is that *the effect of a given treatment upon the potency of a material cannot be arrived at by the comparison of the treated and untreated materials on an equal weight basis.* This may be illustrated by data obtained by H. A. Hill in this laboratory

on the effect of the passage of air through a boiling molasses solution, upon the yeast growth stimulant. The data are plotted in fig. 2. The optimum concentration for the untreated molasses is at 9 per cent. At this concentration the treated material is much less potent than he untreated molasses, while at 8 per cent the reverse is true. In any instance where there is an optimum we may consider the presence of an accelerating and an inhibiting factor. In the case under discussion the inhibiting factor is destroyed by the treatment more rapidly than the accelerating factor. It is conceivable that such a treatment, by removing a noxious material more rapidly than the desirable substance, might actually be a useful method of purification, an idea contrary to the usual procedure of discarding any methods which are destructive to the material sought.

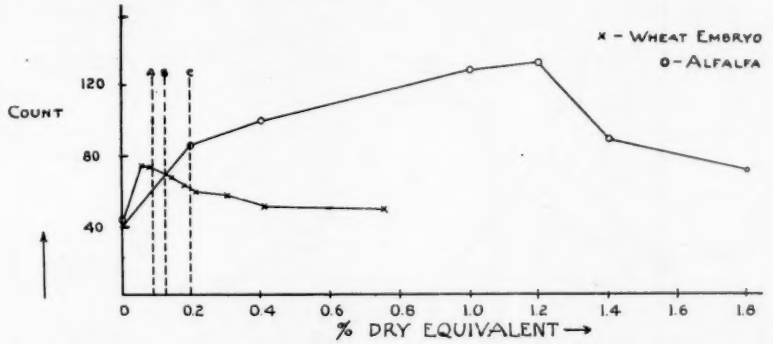

FIG. 1. Influence of extracts of wheat embryos and alfalfa upon the growth of yeast.

The relative potencies of two materials or of two concentrations of the same material as they affect the growth of a microorganism as determined for one temperature, may not be true for any other temperature. It has been shown (**6, 8, 9**) that the optimum concentrations of ammonium chloride for growth is a linear function of the temperature. The concentrations of the other constituents of the medium do not need to be so varied (**14**). Typical data on this point are plotted on fig. 3. It is evident that while a concentration of 0.0375 N ammonium chloride gives a larger crop than 0.0425 N at 35° C. the reverse is true for 42° C.

Since the optimum temperature for the growth of the organism depends not only upon the kinds of materials present in the medium but also upon their concentrations there can be no general meaning to the term "optimum temperature" for growth. If the concentration of the ammonium salt in the medium used is 0.0380 N (fig. 3) the optimum temperature will be at 35° and for 0.0425 N at 42°. It is clear that the "optimum temperature" in-

creases with increase in concentration of the salt. It is of interest to note that larger crops and more rapid growth were obtained at 42° C. than at any other temperature, provided optimum concentrations are maintained; at 43° the growth is abruptly less. It would seem that the ammonium salt acts as a temperature "buffer" permitting growth of the organism at temperatures higher than those considered to be "normal." The crop of yeast in wort at 42° may be increased several hundred per cent by the presence of a 0.0294 N ammonium chloride (8). This fact shows the difficulty in defining the growth of yeast in beer wort as "normal." The improvement is not so great at lower temperatures; at 30° C. the addition of the salt possesses no advantage.

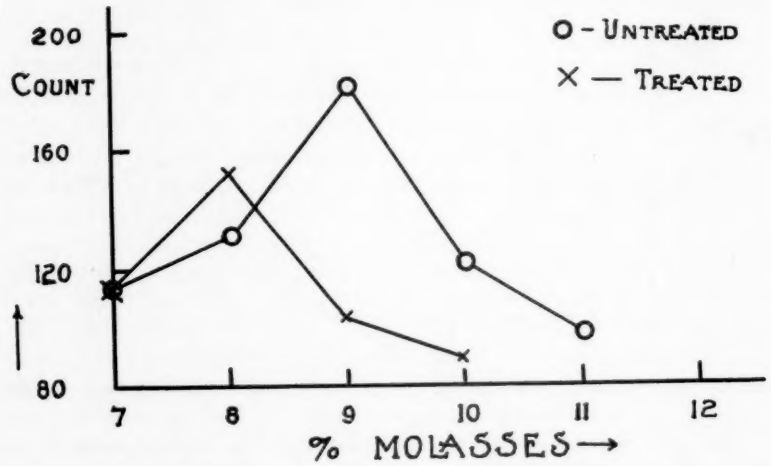

FIG. 2. Influence of aerated and unaerated boiling molasses upon the growth of yeast.

Likewise the "thermal death point" for an organism has no general meaning but is a function of the kinds and concentrations of materials present. It would be expected that the presence of ammonium salt would raise the thermal death point of yeast and experiments are in progress in this laboratory to test this point.

It may also be stated that the temperature coefficient for the growth of a microorganism is a function not only of the components of the medium but of their concentrations. It is apparent in the case of a medium containing ammonium chloride, that raising the temperature of the medium at a given concentration of the salt affects the growth of yeast in the same way as increasing the concentration of the ammonium salt at constant

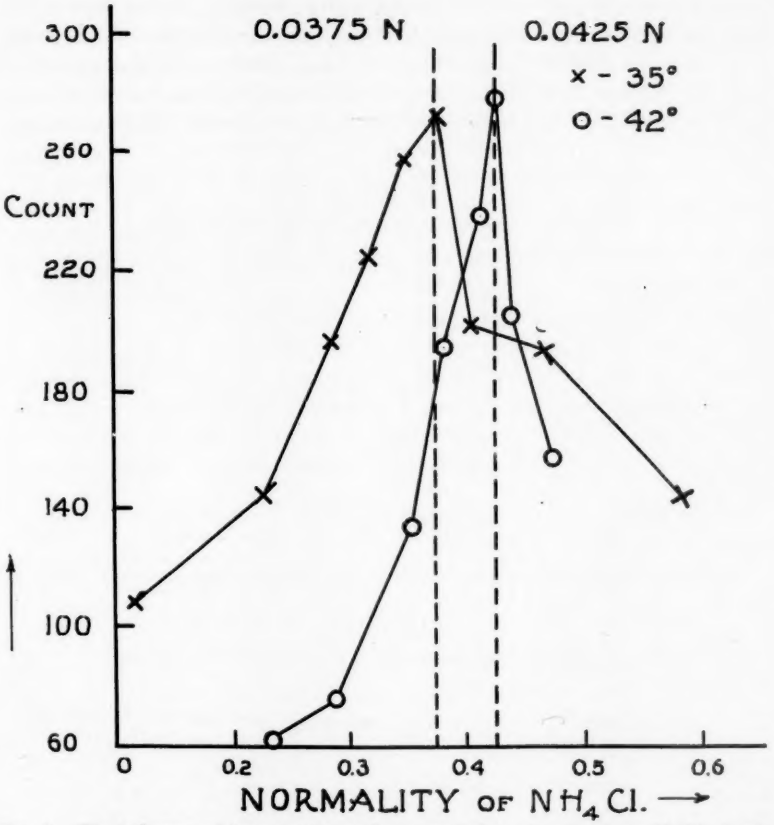

FIG. 3. The influence of temperature, and concentration of ammonium chloride in the medium, upon the growth of yeast.

temperature. As an illustration let us take the medium containing 0.0375 N ammonium chloride, the optimum concentration for 35° C. (see fig. 3). If the growth rates of yeast be determined at 22° and then at 32° C. it is obvious that the growth rate will increase by the rise in temperature and also by the fact that the concentration of the salt is more favorable at 32° than at 22°. The growth rate at 42° will be greater because of rise in temperature but decreased because the concentration of the salt is less favorable at the higher temperature.

The effect of temperature *per se* can be determined only by adjusting the concentrations of the constituents of the medium to optimum conditions

for each temperature. In the following table I are given values for the temperature coefficients for the growth of yeast obtained by SLATOR (**15**) with a medium of constant composition and those obtained in this laboratory with the medium E adjusted to optimum concentrations. A preliminary report of this work has been made (**9**) and the details will soon be published.

TABLE I

TEMPERATURE COEFFICIENTS FOR THE GROWTH OF YEAST

TEMPERATURE IN DEGREES C.	SLATOR	MEDIUM E ADJUSTED TO OPTIMUM CONCENTRATIONS
10	5.6	3.5
15	3.8	(2.8)
20	2.8	2.2
25	2.2	(1.8)
30	1.9	1.5
35	1.6	(1.3)
40	(1.3)	1.0
Variation	4.3	2.5

Average values obtained by various investigators are shown in table II.

TABLE II

AVERAGE TEMPERATURE COEFFICIENTS FOR THE GROWTH OF YEAST

INVESTIGATOR	TEMPERATURE RANGE	AVERAGE TEMPERATURE COEFFICIENT
Slator (**15**)	10–35	2.97
Herzog (**12**)	14–28	2.88
Aberson (**1**)	12–33	2.72
Sherwood and Fulmer (**14**) W_0	10–40	2.18
W_1	10–40	1.90
E_1	10–40	2.05

W_0 = wort without ammonium chloride.
W_1 = wort with optimum concentration of ammonium chloride.
E_1 = medium E optimum per each temperature.

It is to be noted that the values obtained in this laboratory are considerably different in value and range than those obtained without appropriate variation in the concentrations of the constituents of the medium.

Two materials can not be compared as a source of a protoplasm constituent on the basis of their effect upon the growth rate of the microorgan-

ism unless secondary effects are compensated in a series. In ammonium chloride we have a case in which a salt serves the dual purpose of furnishing a protoplasmic constituent, nitrogen, and of influencing the growth by affecting the physical chemical environment. The generalization has been made (**8**) that "if the addition of ammonium salt to a medium increases its ability to dehydrate wheat gluten, the addition will likewise improve the medium for the growth of yeast, the effect being maximum for any combination in which the gluten is least swollen." The increase in yeast crop upon the addition of the salt to beer wort must be due almost wholly to the physical chemical effect rather than to the capacity of the salt as a source of nitrogen.

Comparisons of two materials as they affect the growth of a microorganism at one growth phase may not apply at any other growth phase. While seven growth phases have been pointed out by BUCHANAN (**2**) only two will be discussed here, the logarithmic or phase of maximum growth rate, and the maximum stationary phase or total "crop." Materials as they affect these two phases may be classified as in table III.

TABLE III

LOGARITHMIC PHASE	TOTAL CROP
I—Increase	No effect
II—Increase	Decrease
III—Increase	Increase
IV—Decrease	No effect
V—Decrease	Decrease
VI—Decrease	Increase
VII—No effect	No effect
VIII—No effect	Decrease
IX—No effect	Increase

A consideration of the nine cases shows the necessity of indicating in each instance the particular growth phase involved in growth studies. In the industrial production of microorganisms or their products where time, with accompanying overhead, is the important element it is the logarithmic phase that is the point of interest and the total crop may not be important. In other instances time is not an important element and the increase in total crop is the goal. The effect of ammonium chloride upon the growth of yeast falls into type III or V depending upon whether the concentration of the salt is greater or less than the optimum for the given temperature.

It has been shown by the writer (**10**) and by CLARK (**4**) that the point of inflection of the growth curve, *i.e.*, the point at which the logarithmic

phase ceases, coincides with a definite concentration of alcohol and that certain properties of the cell change abruptly at this point. For example, (**10**) the time required for 100 per cent. of the yeast cells to become permeable to methylene blue in the presence of 0.65 per cent. phenol at 25° C. in terms of carbon dioxide produced are shown in fig. 4. By the use of such a relationship it is possible to compare the effects of toxic agents upon yeast on a quantitative basis, by always using the yeast before the alcohol reaches the critical concentration.

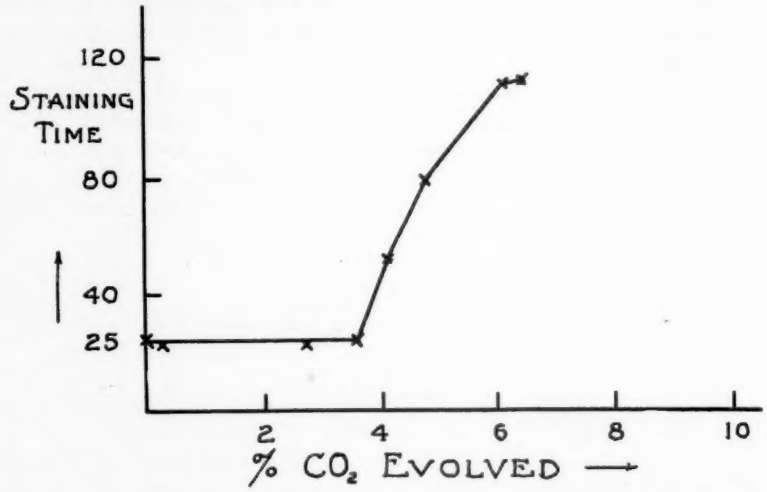

FIG. 4. The relation of staining time for methylene blue, to permeability of yeast cells, as measured by CO_2 production.

An increase in total crop will result by any process leading to a removal of the alcohol or other toxic products from the sphere of action, a fact in harmony with the law of mass action. This may be accomplished by the process of aeration, by electro-dialysis, by precipitation, by the formation of non-toxic soluble compounds, by decreasing the chemical potential (**10, 13, 5, 3**), by the addition of some non-toxic material in a fine degree of dispersion, in which the toxic agent is more soluble than in the medium, or by the use of adsorbents and the like.

Results obtained by one criterion of toxicity are not necessarily applicable to any other criterion. The usual criterion of toxicity of an agent to a microorganism is its effect upon the ability of the cell to reproduce when replaced in a favorable environment, *i.e.*, in causing the death of the cell. This is an instance of negative growth from the viewpoint of repro-

duction. Several criteria of toxicity, or cell injury, which have been used were previously reviewed (**11**). Such criteria are the effect upon reproduction, electrical conductivity, permeability, rates of metabolism, and stainability by various dyestuffs.

It was found by FRASER (**5**) and by the author (**10**) in the study of the stainability of yeast by methylene blue that the cells died, that is, failed to reproduce in a favorable environment, a considerable time before they would be stained by the dye. Moreover, the increase in toxicity of the phenol by the presence of sodium chloride or by ethyl alcohol was directly proportional to the increase in the chemical potential of the phenol. We have here an illustration of the fact that toxicity data obtained by various criteria of cell injury are not necessarily analogous to the effect upon reproduction and hence their graphs cannot be called "death" curves. In toxicity studies, the particular criterion of toxicity used must be emphasized.

Summary

The following general principles with several corollaries are formulated and illustrated with reference to the study of the growth of microorganisms.

1. The relative potencies of two materials as they affect the growth of a microorganism cannot be arrived at by the comparison of the effect of equal weights or concentrations of materials.

2. The relative potencies of two materials or of two concentrations of the same material as they affect the growth of a microorganism for one temperature may not be true for any other temperature. Hence the terms "optimum temperature," "thermal death point," "temperature coefficient of growth" have no general meaning but are functions not only of the components of the medium but also of their concentrations.

3. Two materials cannot be compared as a source of a protoplasmic constituent on the basis of their effect upon the growth rate of a microorganism unless secondary effects are compensated in a series.

4. Comparisons of two materials as they affect the growth of microorganisms at one growth phase may not apply to any other growth phase.

5. Results obtained by means of one criterion of toxicity are not necessarily applicable to any other criterion.

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SULPHUR IN RAINWATER

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(WITH ONE FIGURE)

Introduction

During the last few years considerable data have accumulated concerning the sulphur content of rainwater. No doubt much of the interest in this subject has arisen because of a desire to ascertain how far the sulphur in rainwater would go in making up what many thought was a present or coming agricultural deficiency of this element in the soil. In table I we have summarized some of the data on this subject.

The data of table 1 show a great variation in the sulphur content of rainwater falling at different places. The influence of coal consumption is clearly shown. For example, the sulphur content of rainwater falling at an industrial city such as Leeds, England, or Knoxville, Tenn., is much greater than that of a purely agricultural section such as Ames, Ia. The data for Mt. Vernon, Ia., show that the amount of sulphur brought to the soil by the rain may vary decidedly from year to year. The data of table I will be referred to again in connection with the discussion.

During the years 1921–1923 we made analyses for sulphur of rainwater collected at Chicago, Ill., and near Liberty, Indiana. The station in Chicago was located at the University of Chicago campus. Liberty is a town of about 1,300 inhabitants, with hardly any manufacturing. The station was located about four miles northeast of the town. Richmond, a town of about 27,000 population and with considerable manufacturing, is located about thirteen miles slightly west of north of the station. Thus the data should give a good comparison of a great industrial and residential center, and a typical agricultural section.

Method

The water was collected in an ordinary eight-inch rain gauge such as is used by the U. S. Weather Bureau. The collecting surface is eight inches in diameter. The water is measured in a small central cylinder of such a size that the amount of water falling on the eight-inch surface is magnified ten times. So a small rainfall can be accurately measured. For the collection of snow the lid was left off and the snow was collected in the large cylinder. It was melted and the water poured into the small cylinder for

TABLE I

RESULTS OF PREVIOUS INVESTIGATIONS

INVESTIGATOR	PLACE	RAINFALL IN INCHES	YEAR	LBS. S PER ACRE PER YEAR
Crowther and Ruston (3)	Leeds, Eng.	.....	Nov., 1907–Oct., 1908	134.4–29.2
Crowther and Ruston (3)	Garforth, Eng.	26.95	Oct., 1906–Dec., 1909	38.2
Crowther and Steurt (4)	Near Leeds	25.8–33.2	July, 1911–June, 1912	152–51.2
Erdman (8)	Near Ames, Ia.	30.38	1921	14.89
MacIntire and Young (14)	Knoxville, Tenn., center of city	13.11	1915–1921	94.5
	1½ miles from center of city	14.45	1913–1921	51.5
MacIntire and Young (14)	7 miles from city	14.90	1919–1920	18.6
MacIntire and Young (14)	Other points in Tenn.	14.95–16.01	1919–1921	234–12.7
Stewart (19)	Urbana, Ill.	.....	1913–1919	45.1
Wilson (22)	Ithaca, N. Y.	27.15	1918–1920	26.19
Powers (17)	Corvallis, Ore.	25.40	Nov., 1920–Feb., 1921	1.473
Hart and Peterson (11)	Madison, Wis.	10.7	June, 1910–Oct., 1910	4.28
Artis (1)	Mt. Vernon, Ia.	18.49	Oct. 12, 1914–June 19, 1915	1.965
Peck (16)	Mt. Vernon, Ia.	17.69	Oct. 20, 1916–June 8, 1917	3.374
Trieschman (21)	Mt. Vernon, Ia.	22.25	Oct. 1, 1918–June 15, 1919	.603
———	Mt. Vernon, Ia.	13.44	Oct. 9, 1919–May 22, 1920	20.412
Shaffer (18)	Mt. Vernon, Ia.	20.97	Aug. 18, 1920–June 1, 1921	40.83
Fries (10)	Mt. Vernon, Ia.	17.46	Sept. 19, 1921–June 2, 1922	.276
Miller (15)	Rothamsted, Eng.	29.95	April, 1881–March, '87	6.96
Johnson (12)	Points in Ken.	41.37–45.60	April 1, 1921–April 1, 1923	17.10–41.19

measurement. In this paper the word "rainwater" is used to include both rain and melted snow. In the first measurements a wooden rule was used. But the tendency of the water to crawl up the stick made accurate reading difficult, so the final practice adopted was to divide the number of cc. collected in a month by the cc. in an inch of rain, over a surface eight inches in diameter. The figures in tables I and II for the inches of rain each month, have all been corrected on this basis. The water was usually col-

lected after each rain. It was stored in stoppered glass bottles until the analysis was made. Each month's rainfall was analyzed separately. Above each rain gauge a wire netting was strung in order to prevent contamination from birds.

TABLE II

SULPHUR IN RAINWATER — LIBERTY, INDIANA

Month	1921–1922 Rain Inches	1921–1922 Total S lbs. per A	1921–1922 Mgs.$BaSO_4$ per L	1922–1923 Rain Inches	1922–1923 Total S lbs. per A	1922–1923 Mgs.$BaSO_4$ per L
March	8.12	5.411	19.8	5.85	.....	.....
April	5.58	2.920	16.8	6.89	4.893	22.8
May	2.59	1.757	21.8	3.32	1.780	17.2
June	3.48	2.037	18.8	3.01	1.276	13.6
July	1.51	0.924	19.6	3.45	1.376	12.8
Aug.	6.95	2.423	11.2	5.50	0.855	5.00
Sept.	5.67	1.941	11.0	6.11	2.603	12.8
Oct.	4.06	2.607	20.6	4.33	2.969	22.0
Nov.	5.43	3.517	20.8	1.88	2.809	48.0
Dec.	2.36	4.618	62.8	3.81	9.630	81.2
Jan.	2.65	11.256	136.4	3.79	16.614	140.8
Feb.	0.94	6.634	225.2	1.01	9.150	288.4
Totals	49.34	46.045		48.95	53.955	(11 months)

The method of total sulphur determination was the bromine-nitric-acid method which is outlined in a general way by HART and PETERSON (**11**). A measured amount of the rainwater, the amount depending upon the sulphur content, was evaporated to dryness in a glass dish. Twelve drops of bromine water were added before starting the evaporation and this was repeated twice as the water became more concentrated. Twelve drops of bromine water and the same amount of nitric acid were added to the dry residue and the evaporation was repeated. The dry residue was taken up in dilute hydrochloric acid and then evaporated to dryness, this process being repeated twice in order to get rid of all nitric acid. The soluble part of the residue was taken up in water. The solution was filtered and the filter washed until the filtrate was neutral. The filtrate was diluted to 200 cc., heated to boiling, and the sulphates precipitated out by adding 10 cc. of hot barium chloride solution to the boiling solution. The beaker containing the barium sulphate was allowed to stand on the steam bath over night and at room temperature for the remainder of the twenty-four hours. The barium sulphate was filtered off, washed free of chlorides, allowed to dry on the filter, and the filter with the precipitate was ignited in a muffle furnace. After cooling in a desiccator the precipitate was weighed.

Data

Tables II and III and fig. 1 give the results of the analyses. For the year 1922–1923 the record for the total sulphur is not complete. The influence of coal consumption is clearly shown. In the case of the year 1921–1922, for which we have complete data for the total sulphur, over five times as much sulphur was brought to the ground at Chicago as at Liberty. The largest amount of sulphur was brought to the soil in January in the case of the Liberty rainwater and in November in the case of Chicago. For Liberty this corresponds with the lowest average mean monthly temperature as determined by the Weather Bureau station at Richmond, thirteen miles west of north of the station. Of course the temperature record for Liberty may have been different, but there is no Weather Bureau station there. The lowest average mean monthly temperature for Chicago as determined by the Weather Bureau station on the University of Chicago campus also was in January. The fact that the maximum amount of sulphur was found in the rainwater of November rather than January probably may be accounted for by the much greater rainfall in November as compared with January. The highest percentage of sulphur content comes in February for both Chicago and Liberty, but it is to be noticed that the rainfall for February is very low in each case. The minimum amount of sulphur comes in July for Liberty and in June for Chicago. The influence of coal consumption is perhaps even more clearly shown by the summary in table IV, and by fig. 1. The largest amount of sulphur is found in the winter months. There is a decrease in the spring, a minimum amount in the summer, and then an increase in the autumn.

TABLE III

	Sulphur in rainwater 1921–1922			Chicago 1922–1923		
Month	Rain Inches	Total S lbs. per A	Mgs.$BaSO_4$ per L	Rain Inches	Total S lbs. per A	Mgs.$BaSO_4$ per L
March	4.97	32.914	212.0	4.32	30.401	226.0
April	4.58	12.262	86.0	4.05	19.823	157.0
May	0.91	2.641	93.0	5.28	20.071	122.0
June	1.76	1.919	35.0	0.027	0.900	156.0
July	2.40	6.272	84.0	3.40	4.274	42.0
Aug.	5.45	11.798	69.0	1.18	7.233	197.0
Sept.	4.52	15.480	110.0	3.68	8.948	78.0
Oct.	3.45	20.631	192.0	3.68	22.014	192.0
Nov.	4.56	42.722	301.0	2.72	28.151	332.0
Dec.	2.69	20.346	243.0	.....	.....	.....
Jan.	1.03	33.276	1037.0	.....	.....	.....
Feb.	0.49	27.191	1744.0	.....	.....	.....
Totals	36.81	227.452		28.33 (9 months)	141.815	

Discussion

Factors affecting the sulphur content of rain-water

Amount of coal consumed.—The amount of coal consumed in any region is the main factor affecting the sulphur content of the rain-water of that region. This is shown to be true by the correlation between coal consumption and the amount of sulphur in the rain-water. Near large industrial and residential cities, the sulphur content of the rain-water is high, while as the distance from the city increases there is less and less sulphur present. Also there is much more sulphur in the winter rain-water than in that of the summer. The data as given in tables II, III, and IV, and fig. 1 illustrate these points well. For the year 1921–1922 there was over five times as much sulphur in the Chicago rain-water as in that at Liberty. And the data as summarized in table IV show quite a seasonal variation at each place.

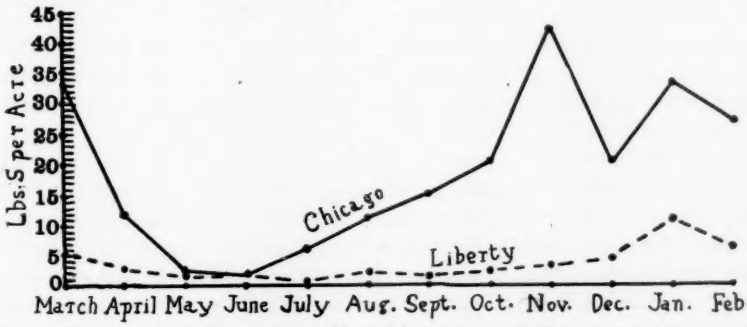

Fig. 1. Curves showing the total monthly sulphur content of Chicago and Liberty rain-water for the year 1921–1922.

The effect of coal consumption on the sulphur content of rain-water has been noted by a number of investigators. MacIntire and Young (**14**), find in the annual precipitation at the center of the city of Nashville 94.5 pounds of sulphur, 1.5 miles from the center 51.5 pounds, and 7 miles from Knoxville 18.6 pounds. The results of Crowther and Ruston (**3**), for stations at different points in the city of Leeds, that of Crowther and Steurt (**4**), for stations outside the city at different distances from it, and that of Johnson (**12**), for different points in Kentucky also illustrate the same point.

A number of workers have found more sulphur in the rain-water during the winter months (**14, 12, 18**). Coal varies widely in its sulphur content. The kind of coal consumed no doubt affects decidedly the sulphur content of rain-water. Shaffer (**18**) explains the unusually large amount of sul-

TABLE IV

TOTAL SULPHUR BY SEASONS (1921–1922)

	WINTER Dec., Jan., Feb.	SPRING March, April, May	SUMMER June, July, Aug.	AUTUMN Sept., Oct., Nov.
Liberty Total S.	22.508	10.088	5.348	8.065
Chicago Total S.	80.813	47.817	19.989	78.833

phur in the rain-water at Mt. Vernon, Iowa, during 1920–1921 by the fact that the price of coal forced the people to use cheaper grades.

Our data for Liberty shows a higher sulphur content than most workers have obtained for purely rural sections. For example, ERDMAN's (**8**) data for Iowa may be taken as typical for a purely agricultural region. The annual precipitation at Liberty contains over three times as much sulphur as that at Ames, Iowa. But if everything is considered, this is perhaps not surprising. In 1920 Indiana had a population of 80 per sq. mile, Iowa 42. In 1921 Indiana had 109 manufacturing establishments with a product valued at $1,377,772,000 annually, whereas Iowa had 3,527 with an output valued at $521,446,000 annually. ERDMAN (**8**) reports a rainfall of 30.38 for the year, while the rainfall at Liberty for 1921–1922 amounted to 49.34 inches. The great seasonal variation in the sulphur content of the rain-water at Liberty certainly indicates that coal consuption is the main source of the sulphur, while as indicated below this factor may not be so important at Ames.

EVOLUTION OF HYDROGEN SULPHIDE.—The sulphur content of the rain-water of purely rural sections as shown by various workers may not vary much with the season, or may be greater in the summer than winter. STEWART (**19**), in the case of the rain-water at Urbana, did not find any great seasonal variation in its sulphur content. CROWTHER and RUSTON found that the precipitation from April to September contained more sulphur than that from October to March. MILLER (**15**), in his summary of the sulphuric acid in Rothamsted rain for the two periods April to September, and October to March, shows that the amount is practically the same for the two periods. ERDMAN (**9**), found that more sulphur was brought to the soil by the rain during the growing season March to October than during the winter. He explains this on the basis of the more rapid production of hydrogen sulphide by bacteria during the summer. Others have emphasized the importance of the decay of organic matter as a

source of the sulphur in rain-water. MACINTIRE and YOUNG (**14**) refer to articles by LAWES, GILBERT, and WARINGTON. These authors refer to the opinions of ANGUS SMITH that the sulphuric acid in rain-water is largely the result of the decay of animal and vegetable matter.

It may well be that in rural districts where not much soft coal is consumed, sulphur compounds resulting from the decay of organic matter may be more important as a source of the sulphur in rain-water than coal consumption. In such cases there would not be the great seasonal variation which was noted in the case in which coal consumption accounts for more of the sulphur. Some of our determinations of the sulphate sulphur showed more of the sulphur present in the sulphate form at both stations during the winter than during the summer, and more present at all seasons in Chicago rain than in Liberty rain. This is what one would expect if the evolution of hydrogen sulphide is of much importance in accounting for the sulphur content of rain-water.

RAINFALL.—It would be expected that the amount of sulphur brought to the sail by rain would vary directly with the amount of rainfall. But the distribution of the rain is probably important. A certain amount of rain coming in a cloudburst would be expected to bring less sulphur to the soil than the same amount coming in drizzling rains over a long period of time, because in the latter case the atmosphere is kept cleaner of sulphur and less is lost by being carried away by air currents. As already stated, the fact that in the case of the Chicago rain-water the largest amount of sulphur was brought to the soil in November while the lowest average mean monthly temperature was in January may probably be accounted for by the much larger rainfall in the month of November as compared with January. But while the total sulphur brought to the soil is greater in the case of a large rainfall, the percentage sulphur content of the water is greater in the case of a small rainfall. The small rainfall in February, 1922, both at Liberty and Chicago, probably accounts for the greatest percentage sulphur content as given in the column "mgs. $BaSO_4$ per liter" coming in February rather than in January, the month of the lowest average mean monthly temperature at both places. In June, 1922, there was very little rain at the station in Chicago. The result was an unusually high percentage sulphur content of the rain that fell.

LOCATION WITH RESPECT TO THE PREVAILING WINDS.—We did not have enough stations to give any data on this point, but others have clearly shown its importance. CROWTHER and STEURT (**4**) established stations around Leeds at different distances from the city. In passing from the city northwards or northeastwards there was a more rapid decrease in the

sulphur content of the rain-water than in passing eastwards. Thus is shown the influence of the westerly prevailing winds.

Agricultural significance of the sulphur in rain-water

It seems correct to state that theoretically we have as serious a sulphur problem in the United States as we have phosphorus problems, and it is generally agreed that phosphorus is commonly deficient in our soils. There is less sulphur than phosphorus in most agricultural soils. Farm crops contain on the average about as much sulphur as phosphorus. And while rain-water contains considerable sulphur, yet much more sulphur is lost in drainage than is added to the soil by the rain-water.

In the actual use of sulphur fertilizers very greatly increased yields have been secured in the Northwest, Oregon, Washington, and Idaho. In some other sections of the United States beneficial results from the use of sulphur-containing fertilizers have been noted. The yield of cotton has been materially increased, and very greatly increased yields of sugar cane in Cuba have been secured. Yet it seems fair at the present time, to state that the sulphur problem has not developed to anything like the extent expected by many a few years ago.

Geologists estimated that in 1920 the United States had upwards of 1,400,000 million tons of various grades of true bituminous coals, 49,863 million tons of semi-bituminous, 987,514 million tons of sub-bituminous and 1,093,290 million tons of lignite (**6**). Coal varies widely in its sulphur content. The Encyclopaedia Americana gives analyses of coal of different parts of the United States. The sulphur content of bituminous coal, according to the table, ranges from 0.1190 to 3.8390 per cent, and the percentage given for semi-bituminous is 0.79 per cent. Some bituminous coal has a higher sulphur content than is indicated by these figures. Over 500,000,000 tons of bituminous coal are consumed in the United States in a year. And while according to some authorities only one eighth to one fourth of the sulphur of the coal is volatilized (**7**), it may be seen that a large amount of sulphur is each year given off into the air by the burning of bituminous coal .The consumption of anthracite coal increases this amount though anthracite contains on the average much less sulphur than the bituminous. The rain may bring the soil much of this sulphur.

We have then in the sulphur in coal a source of sulphur in addition to that naturally present in the soil. In agricultural regions of large rainfall, the sulphur in rain-water may at least delay the development of an acute sulphur deficiency in the soil. Increased rainfall means of course increased loss of sulphur in drainage. But cropping decreases this loss in drainage, and legumes, of course, tie up much more of the sulphur than non-legumes.

The fact that the legumes used for pasture make considerable growth early in the season when the sulphur content of the rain-water is higher, is an advantage. It has been shown that soils in alfalfa have a higher sulphur content than soils in grain (**20**). And in a previous publication (**5**), the senior author found in the case of two soil samples taken in a field which had been in clover once in four years, that the sample from the earth of the field supporting a good stand of clover contained almost twice as much sulphur as the sample from a part of the field on which little clover grew. Of course the roots of clover and alfalfa may bring up from the subsoil considerable sulphur, but the sulphur of rain-water is also no doubt made use of. On the death of the plants, soil bacteria rapidly make the organic sulphur again available for crops.

In this way the sulphur in rain-water in certain cases may materially supplement the sulphur in the soil. But because of the small amount of sulphur present in the soil, the large amount used by crops, and the serious loss of this element in drainage, it would seem that the sulphur of the rain-water could at the most only delay the coming of a serious general sulphur deficiency in the soil. Finally sulphur will probably have to be supplied as a fertilizer if crop yields are to be maintained.

Summary

1. The data clearly show the influence of coal consumption on the amount of sulphur in rain-water. The rain falling at the station near Liberty, a good agricultural region, contained for the year less than one fifth as much sulphur as was found at the Chicago station. At each place there was a marked seasonal variation, the largest amount of sulphur being in the winter rain.

2. In regions where the amount of coal consumed is not great the evolution of hydrogen sulphide into the air may be a matter of considerable importance for the total sulphur brought to the soil by rain.

3. The amount of sulphur added to the soil by rain varied directly with the rainfall, but the percentage sulphur content of the rain varied inversely with the amount of rain falling.

4. In agricultural regions where much soft coal is burned the sulphur of rain-water may materially supplement the sulphur present in the soil and thus delay the coming of an acute sulphur deficiency in the soil. But that this can be only a delay is indicated by the small amount of sulphur present in the soil, the large amount lost in drainage, and the large amount used by crops.

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BRIEF PAPERS

A PLANT PHOTOMETER

Light may influence the growth of plants in at least three possible ways: by its intensity, its duration, and its quality or wave length. In experiments dealing with various plant responses to light these three factors should be considered, and if not controlled, a careful and complete description of the experimental conditions should be stated in order to aid in interpretation of data by others interested. A study of any one of these factors is always complicated by the presence of the other two, thus making it difficult to evaluate the relationship of each to the plant process under investigation. In this paper a method of studying the quality or wave length of light as it affects plant growth in phototropic bending is briefly described.

The apparatus, which may be called a plant photometer, consists of a box (30 x 28 cm. and 350 cm. long) divided into three compartments, each painted a dead black. The central compartment is 100 cm. long and the partitions separating it from the end compartments are fitted with centrally located windows of a size to hold 2 x 2-inch Wratten light filters. The end compartments contain electric lamps so arranged that they can be moved easily toward or away from the windows.

A seedling growing in a container placed in the central compartment, equidistant from equally intense lights of the same wave length will grow erect, but if one of the lamps is removed a few centimeters more distant from the plant the seedling will then grow toward the nearer one, that is, toward the more intense light since the intensity varies inversely as the square of the distance. Some plants easily distinguish between lights of different intensities and may be employed as the adjustable indicator or photometer screen in the ordinary Bunsen photometer, only that the time involved in determining the relative intensities of two lights is considerably longer in the case of the plant indicators.

The question arises as to which way a seedling will grow when the lights are of the same intensity but of different wave lengths. An actual experiment with a given plant will serve to illustrate the method and answer this question for a specific set of conditions. Two Wratten light filters, number 24 (red) and number 61 (green) were used. According to published data, the total percentages of light transmitted by these filters are 24 and 18 respectively. A 25-watt, frosted, round bulb Mazda lamp was placed back of each filter at such a calculated distance as to ensure light of equal intensity reaching the center of the central or plant compartment. A buck-

wheat seedling growing in a small water culture container was placed midway between the two light filters. The container was rotated to a position in which the plant stem was exactly perpendicular to a straight line joining the two lamps. The box was closed and after a few hours the plant was examined and found to have grown toward the green light.

Since there is always the possibility of two similar electric lights differing in intensity enough to be detected by the plant they should be interchanged and the experiment repeated with another seedling. To insure more accurate results the intensity of light coming through each filter should be tested by means of a suitable thermopile and a galvanometer. If the intensities differ, one light should be moved to such a position that the galvanometer shows the same deflection for each light when the thermopile is placed at equal distances from the light filters. In the experiment noted above the calculated distances were corrected to those obtained by actual intensity measurements.

It is not the purpose of this paper to set forth results regarding the action of various light waves on growth but merely to indicate a method which promises to give quantitative results. This is especially true since the methods of measuring light have become more refined and a number of very good light filters are available which limit the wave-lengths to definite portion of the spectrum.—Earl S. Johnston, *University of Maryland.*

SCIENTIFIC PUBLICATION

In the interests of economy of time and money it is very desirable to make scientific papers as brief as clearness of presentation permits. The output of scientific papers grows constantly, and it is increasingly difficult to keep abreast of the literature in any important field. Moreover, the costs of publication have become so high that authors should be willing to condense the presentation of their results as much as possible. Many papers would be greatly improved by more careful organization, and by concentrating upon the important features.

With reference to organization, writers can enhance the value of their work by a little attention to the order of presentation. Papers that have a brief and pointed approach to the problem, succinct statements of the methods used, clear cut presentation of data, and critical discussion of the problem as a whole, are usually briefer and more desirable than more diffuse papers in which no attempt has been made at orderly presentation.

As to the expensiveness of publication, the main difficulties arise in connection with the use of graphs, plates, and tabulated data. The physiologist must use these devices, but when they are used in excess of the real requirements, they add enormously to the costs of publishing. It is wise,

therefore, to restrict the use of such material as closely as adequate presentation will permit. It costs more than twice as much for a page of tabulated data, for instance, as for a page of text discussion. If writers were to select their material carefully, so as to illustrate their discoveries adequately, but not extravagantly, it would be much easier to cover the costs of publication. We hope that contributors to PLANT PHYSIOLOGY will remember these facts when they are preparing manuscript for its pages.

Frequently graphs are not made in the right proportion for reproduction. It is customary to reduce the size of a graph to about one third of the original size. The whole graph must then be made three times as large as it is to appear, the lines three times as thick, the figures and letters three times as large as they are to appear on the printed page. If the numbers and letters are too small, they become illegible on reproduction at reduced size. Careful attention to uniformity of size of figures and letters used to designate parts of the graphs will also help to give the final reproduction a good appearance.

The size of the journal page should always be kept in mind in preparing graphs, and plates, and tables. All of them should be so constructed that they will fit the pages of the journal when the reductions have been made.

Many authors make the mistake of placing the legends for graphs and plates on the margin of the drawing paper, or on an attached folder. As the legends must be sent to the typesetters, and cuts to the engravers, this practice entails unnecessary work on the part of the editor. The legends should be typewritten upon a separate sheet, numbered to correspond with the graphs and plates, which should bear numbers on their backs, and the name of the author.

In the preparation of literature lists, contributors are requested to use alphabetical order, and to adopt the style of citation employed in this number, name of author, title of paper, name of journal properly abbreviated, volume number, limiting pages, and year of publication. It will lighten the work, and expedite the issuance of the journal if all of our contributors will remember these simple rules. Such cooperation will be very greatly appreciated.—C. A. SHULL, *Editor-in-Chief*.

NOTES

The Kansas City Meeting of the American Association for the Advancement of Science and the various affiliated and associated societies was well attended, and as in previous years botanists profited by holding their meetings at the same time and place. The arrangements were for the most part very satisfactory, and those in attendance were well pleased with the accommodations provided.

The second annual meeting of the American Society of Plant Physiologists was a very enthusiastic one, full of inspiration to the large numbers in attendance at the meeting. A fine feature was the keen interest of a widely diversified group in the establishment of the journal, and in the program of varied service which the society hopes to render to the plant physiologists of America. From time to time brief notes regarding the progress of these plans will appear in this section of PLANT PHYSIOLOGY. Dr. W. E. Tottingham, of the University of Wisconsin, is chairman of the committee working on standardization of methods of preservation and analysis of plant tissues. Dr. A. L. Bakke, of Iowa State College, is in charge of the work of formulating policies with reference to research and other activities which are to be considered by the society from time to time. Several other committees are at work, and the reports of progress were received with much interest.

The splendid success of this meeting should encourage all of us to plan to attend the summer meeting proposed to be held at Minneapolis in the early summer, and to make the Philadelphia meeting a very notable one.

The International Congress of Plant Sciences to be held at Cornell University, Ithaca, N. Y., August 16–23, 1926, will be a very important meeting, and should be strongly supported by American botanists. It is an enormous task to organize and carry through the arrangements for such a meeting. We can all show our appreciation, and help to make the meeting a success by attending one or more of the sectional meetings. The plans for the Physiology Section meetings are in the able hands of Dr. O. F. Curtis, of Cornell University. The Plant Physiologists of the United States and Canada should take advantage of the opportunity, and take part in the round table discussions.

Fruit Growing.—Under this title Chandler has prepared a book which is an outstanding contribution to horticultural and physiological literature. It is not a textbook, primarily, but a source book of information which con-

tains the observations and experiences of a master workman. It is written in the critical spirit of a true scientist, and will stimulate a great deal of thinking and investigation among its readers. It is a book that one will read several times, and consult again and again for its point of view, and its splendid critical presentation of facts and theories. The author is to be congratulated on having given us the best book so far written on this subject.

The book contains twenty-seven chapters, and has an extensive bibliography selected to accompany the various chapters. The publishers are Houghton Mifflin Co., New York.

Textbook of Plant Physiology.—Attention is called to Lepeschkin's Lehrbuch der Pflanzenphysiologie published by Springer, Berlin, 1925. The book is divided into three sections, the physiology of metabolism, growth phenomena, and movements of plants. The author attempts to ground the discussion in the principles of physics and chemistry, and to give a presentation of the outstanding facts and the most important contributions, rather than a complete survey of the literature, and minute details. Those who have mastered their German will find the book profitable reading.

Photosynthesis.—A book on this subject by Stiles will be welcomed by plant physiologists generally. It is obtainable from Longmans, Green and Co., and should be placed in all of our libraries. An extensive literature has been summarized in excellent fashion, and the American and English sources are given adequate treatment. There are twelve chapters, covering all of the important phases of the process. Chapter X particularly reminds us of how little we actually know about the mechanism of photosynthesis, for the author summarizes our position at present by quoting in the closing sentences, Sachs's statement of more than forty years ago. The mechanism has baffled the greatest chemists of all time, and the most important natural synthetic process, aside from the building of protoplasm, remains a mystery.

Seeds for Investigation.—It is a great convenience to be able to secure seeds of our native plants when we desire to use them for experimental purposes. Sources of material of this kind have been more highly developed in European countries than in the United States. Those who desire seeds of woody shrubs and trees for physiological studies may be able to obtain exactly what they desire from Mr. Conyers B. Fleu, Jr., Ross and Montana Sts., Germantown, Philadelphia, Pa. For a number of years Mr. Fleu has been engaged in handling tree and shrub seeds in quantity. Many of the seeds of forest trees and woody shrubs offer attractive problems in plant physiology, as scant attention has been paid to the physiology of forest trees and shrubs.